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Analysis of Residual Furan in Human Blood Using Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS)

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Abstract For an accurate risk assessment of furan, a potential human carcinogen, levels must be determined in human blood plasma using a simple and robust assay. In this study, solid phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS) was used to analyze blood plasma levels of furan in 100 healthy individuals who consumed a normal diet. The subjects were 30 to 70 years of age and 51% were women. Ultimately, an analytical method was established for analyzing furan in human blood. The limit of quantification (LOQ) and furan recovery rate in blood were 1.0 ppb and 104%, respectively. Finally, furan was detected in 21 individuals (13 males, 8 females) with levels ranging up to 17.86 ppb (ng furan/g food).

Keywords: furan, human blood, solid phase microextraction (SPME), gas chromatography/mass spectrometry (GC/MS)

Introduction

Furan is a colorless chemical (C_4H_4O) with a 5-member ring structure. It is highly volatile due to its low boiling point of 31.4°C and molecular weight of 68. Furan has commonly been used as a solvent in the chemical manufacturing industry (1). It is frequently classified as a parent compound of various derivative compounds known as ‘furans’; in this study, however, we only focused on the parent compound. Furan is found in a wide variety of processed foods, including coffee, canned meat, baked bread, cooked chicken, and caramel (2-7). Maga (8) reported that furan was present in a number of foods, with the highest levels being found in coffee. Furan is formed in foods during conventional heat treatment procedures such as cooking, canning, and baking. Although many chemical pathways have been reported for furan’s formation in heat-processed foods, the major routes are demonstrated as 3 mechanisms, beginning with either sugars, ascorbic acid, or unsaturated fatty acids (9,10). Furan and its derivatives are one of major compounds formed during the Maillard reaction, and have been used in food and tobacco products as flavoring agents (8,10).

Furan, however, is considered to be a potentially hazardous chemical, and was recently classified as a possible human carcinogen (B2 class) by the International Agency for Research on Cancer (11,12). Serious health problems, including cancer and liver toxicity, were reported in laboratory animals after exposure to high doses of furan. Since 2004, furan has been examined not only as a flavor compound in jarred and canned foods, but also as a novel harmful substance, by research facilities around the world, including the U.S. Food and Drug Administration (FDA). Furan’s toxicological properties and human health effects have been studied in terms of both genotoxic (13,14) and non-genotoxic mechanisms (15-17). In order to assess relevant exposure in populations, procedures for furan analysis in human blood have become essential.

Furan analysis has been performed extensively in foods, especially in coffee, canned products, and baby foods (2-8,10). The U.S. FDA has developed a technique that is capable of detecting and quantitating low levels of furan in foods, involving headspace auto-sampling methods coupled with gas chromatography/mass spectrometry (HS-GC/MS) (2,5,7,11,18). To establish an exposure assessment for furan, the FDA has carried out an expanded survey of furan levels in different foods and beverages (11). Although the FDA method is accurate and robust, it is also time consuming and labor intensive. With this method (HS-GC/MS), a calibration curve is created by applying both the standard addition and internal standard methods. As a result, when using the FDA method to analyze a sample, 7 sample preparations should be carried out. To save analytical time and labor, solid phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS) has been applied to furan analysis (19,20). The SPME-GC/MS method is more convenient and simple than the FDA method. Recently, the potential of SPME for sample preparation was widely recognized. SPME integrates sampling, extraction, pre-concentration, and sample introduction into a single process, resulting in high sample throughput (15). Therefore, we chose the SPME-GC/MS method to analyze furan in this study.

So far, there is no published report of furan levels measured in human blood. One hypothesis we project is that detected levels of furan in human blood could be an index of past furan exposure. For accurate risk assessment

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in humans, simple and robust assays should be used to determine blood furan levels. In this study, SPME-GC/MS was employed and then validated as an analytical method for determining residual furan levels in human blood plasma.

Materials and Methods

Human blood samples From June to September 2005, blood plasma was collected for furan analysis from male and female volunteers at Korea University Anam Hospital (Seoul, Korea), according to a protocol approved by the Institute Review Board. The blood samples were obtained from 100 healthy subjects (49 males, 51 females), ages 30 to 70 years. A dietician confirmed that all volunteers consumed a regular Korean diet and did not eat large amounts of furan-containing food items. The subjects rarely consumed the following: smoked salmon and powdered milk (<1 serving/month); apple juice and spaghetti (<2 servings/week); potatoes and peas (<3 servings/week); coffee (<1 cup/day); and canned or jarred foods (<1 serving/week). Since a database on amounts of furan in the typical foods is not available so far, an actual intake of furan per individual was not shown in this study. Also other factors such as smoking were not considered for actual intake of furan.

The blood samples (10 mL) were collected in ethylenediamine tetraamine acid (EDTA) tubes and immediately put on ice to prevent loss of furan by evaporation. The plasma was isolated by centrifugation (630×g, 10 min, 4°C). The clinical samples were stored at −80°C until furan measurement was performed. Individuals handling the samples wore cryo-gloves cooled in ice and minimized the exposure time at room temperature.

Chemicals Furan (+99%, purity) and d_4-furan (+99%, purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC) grade of water and methanol (HPLC grade) were supplied by J.T. Baker (Phillipsburg, NJ, USA).

Furan and d_4-furan stock solutions were prepared from 2 mg/mL of methanolic solution every 2 week. Using a microliter syringe, the stock solutions were diluted with 10 mL of water to a concentration of 4 μg/mL. All analytes were stored at 4°C in completely filled vials closed with aluminum seals until analysis.

Calibration curve of furan Calibration standards were prepared by injecting various concentrations of aqueous furan solution (0.4 to 3.2 ppb), and a fixed amount of d_4-furan (1.6 ppb) as an internal standard, into a 20-mL headspace vial. The furan concentration was determined by the peak area ratio of furan to d_4-furan in the calibration curve. All samples were prepared in triplicate.

Determinations of limit of detection (LOD) and quantification (LOQ) and furan recovery test To determine LOD and LOQ, furan (0.4 ppb) and d_4-furan (1.6 ppb) were injected 7 times and standard deviations (SD) were calculated. LOD and LOQ were calculated based on following equations (21). The slope was obtained from the relationship between sample concentration and corrected signal.

- $LOQ = 10 \times SD/slope$ (ng/mL)
- $LOD = 3 \times SD/slope$ (ng/mL)

For the furan recovery test, furan (final furan concentration: 3.2 ppb) and d_4-furan (final d_4-furan concentration: 1.6 ppb) were spiked into furan-free blood samples according to the procedure described above. The samples were then prepared for SPME-GC/MS analysis as described below.

Sample preparation for furan analysis In a 20 mL headspace vial, 10 mL of distilled water (HPLC grade, J.T Baker) was added to 0.3 mL of blood plasma. The vials were then spiked with d_4-furan (1.6 ppb). The samples were stored at 4°C in completely filled vials closed with aluminum seals until analysis.

SPME-GC/MS analysis The SPME-GC/MS system used in this study was modified from that presented by Goldmann *et al*. (19). The SPME experiment to extract furan from the human blood plasma was operated with a 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA). The fiber was exposed at 60°C for 20 min under constant agitation (200 rpm) in a 20 mL sample vial. Before use, the fiber was conditioned in the injection port of the gas chromatograph at 250°C for 1 hr. The injection was carried out by desorbing the fiber for 5 min at 250°C. The fiber was baked for 5 min at 250°C before the next extraction to blow out any residual analyte.

An Agilent 6890N Network GC system equipped with an Agilent 5975 Inert Mass Selective detector (Palo Alto, CA, USA) was used for GC/MS analysis. Chromatographic separation was performed on HP-PLOT Q columns (15 m ×0.32 mm, 20 μm film, J & W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 44 cm/sec; the gas chromatograph was operated in splitless mode with the injector maintained at a temperature of 250°C. The following GC oven temperature program was applied: 50°C for 5 min, 25°C/min to 230°C, and 230°C for 2 min. The mass spectrometer was operated in selective-ion monitoring mode (SIM) by recording the currents of the following ions: *m/z* 68 and 39 for furan, and *m/z* 72 and 42 for d_4-furan. The corresponding ion ratios for furan and d_4-furan were determined for each measurement. In SIM mode, full scan electron ionization (EI) data were acquired simultaneously to determine appropriate masses for SIM.

Results and Discussion

Calibration curves, determination of LOD, LOQ, and recovery tests In this study, a simple method for determining furan levels in human blood was developed and validated. The SPME-GC/MS system was modified from the method of Goldmann *et al*. (19) to develop a suitable method for our purpose as well as for adaptation to our equipment. Using a 75-μm CAR/PDMS fiber, the SPME conditions for furan analysis were as follows: extraction temperature of 50°C, sampling time of 20 min, headspace/aqueous volume ratio of 9.7 mL/10.3 mL in a 20-mL glass vial, desorption temperature of 250°C, and desorption time of 5 min.

The linearity of the calibration curve was evaluated by plotting the area ratio of furan/d_4-furan from 0.4 to 3.2 ppb.

Table 1. Validation factors for furan analysis in human blood

		Validation values
Linearity	Range	0.4-3.2 ppb
	Equation	y=0.0594x+0.0484
	Coeff. of determination (R^2)	0.9979
Limit of detection (LOD)		0.3 ppb
Limit of quantification (LOQ)		1.0 ppb
Precision	Coefficient of variation (CV)	5.67%
Accuracy	Recovery (1.6 ppb spiked)	104.1±6.1%

As shown in Table 1, good linearity was obtained with R^2=0.9979 as the coefficient of determination. As described earlier, the blood samples were used to determine LOD and LOQ based on signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively. A LOD of 0.3 ppb and a LOQ of 1.0 ppb were obtained.

These LOD and LOQ values were higher than those reported by Altaki *et al.*(22), who previously reported that the LOD of furan in various foods was sample dependent and ranged from 0.008 to 0.07 ppb, while the LOQ ranged from 0.03 to 0.25 ppb. The FDA reported that their analytical method had an LOQ of approximately 5 ppb for most foods, and an LOQ of 2 ppb for most liquids, including coffee (11). In addition, the European Food Safety Authority (EFSA) reported data that were expressed as ranges over 11 food categories with LOQ values of 1-2 ppb (23). In our study, the LOQ was almost 2-fold lower than the values reported by the other scientists (9), and within the range of those obtained by EFSA (23).

To validate the procedure, we performed a recovery test by adding 3.2 ppb of furan and 1.6 ppb of internal standard (d_4-furan) to blood samples without furan, as described in the materials and methods. The recovery rate of the blood samples was 104.1±6.1%. Crews and Castle (24) reported that the analytical recovery rate of furan for major matrices, brewed coffee and baby food, was consistently better than 90% with an LOD of less than 1 ppb.

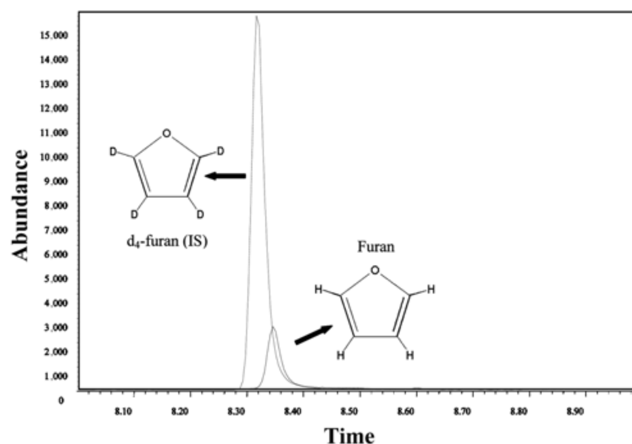

Fig. 1. Typical GC/MS chromatogram of furan in a blood sample.

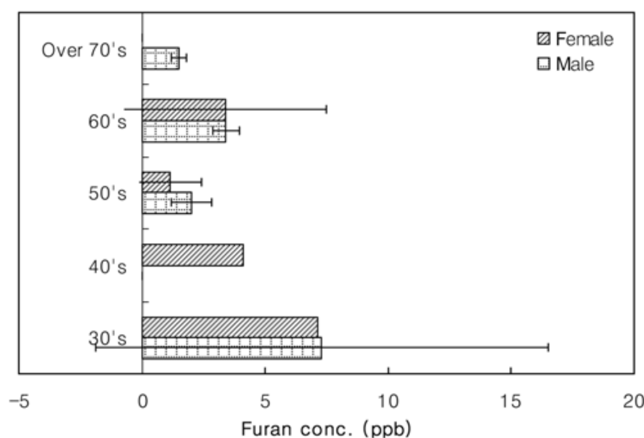

Fig. 2. Furan levels in blood according to sex and age.

Furan levels in human blood samples A total of 100 samples (males: 49, females: 51) were analyzed for furan. The furan concentrations are expressed in ppb and the results according to sex and age are shown in Fig. 2. The samples with estimated furan levels between the LOQ and LOD are reported as <1.0 ppb, and the estimates below 0.3 ppb are indicated as not detected (Table 2).

According to Fig. 2, furan was detected and quantitated at different concentration levels depending on both age and sex. Among the 100 tested samples, furan was detected in 21 samples (males: 13, females: 8) and not detected in 79 samples (males: 36, females: 43). Seventeen blood samples (males: 12, females: 5) were over the LOQ; the highest furan level was 17.86 ppb. The males appear to have slightly higher levels of furan than the females. This is probably mainly due to greater dietary consumption of furan by the males. However it needs more accurate dietary intake data and consideration of other confounding factors such as intake through smoking In Korea smoking rate of male is much higher than that of female.

We previously reported that urinary furan was detected in 56 urine samples (males: 31, females: 25) and ranged up to 3.14 ppb; while it was not detected in 44 urine samples (males: 18, females: 26) (25). In this study, the blood furan level of the males was almost 3.2-fold higher than the level in urine, and the furan level of the females was approximately 3.9-fold higher in blood than in urine. These results suggest that furan is rapidly absorbed from the gastro-intestinal tract into the peripheral blood system, but also excreted in the urine.

After ingestion, furan is rapidly absorbed from the gastro-intestinal tract, metabolized to the reactive multifunctional intermediate *cis*-2-butene-1,4-dial by hepatic cytochrome P450 2E1 (26,27) and then eliminated. *cis*-2-butene-1,4-dial is believed to be a key metabolite involved in furan toxicity and carcinogenesis. Studies show that *cis*-2-butene-1,4-dial forms both protein and nucleoside adducts (26,28); it acts as a mutagen in the Ames assay, and its acute toxic and genotoxic effects are mitigated by glutathione *in vitro* (29). One hypothesis for furan carcinogenicity is that *cis*-2-butene-1,4-dial stimulates cell proliferation, increasing the likelihood of tumor induction (30).

Due to its extremely hazardous nature, we also measured *cis*-2-butene-1,4-dial in the 21 blood samples (males: 13, females: 8) in which furan was detected; however, the compound was not detected in any of the 21 samples. The absence of *cis*-2-butene-1,4-dial does not mean it does not

Table 2. Furan levels in blood as according to sex and age

		Age group					
		30's	40's	50's	60's	70's	Average
Individual		22	23	22	21	12	
Male		11	11	10	11	6	9.8
Female		11	12	12	10	6	10.2
No. of sample	Sex	Age	Furan conc. (ppb)	No. of sample	Sex	Age	Furan conc. (ppb)
1	Male	31	ND[1]	24	Male	54	ND
2	Male	30	ND	25	Male	68	ND
3	Male	58	ND	26	Male	59	ND
4	Male	46	ND	27	Male	74	ND
5	Male	64	ND	28	Male	76	1.20±0.25
6	Male	42	ND	29	Male	66	ND
7	Male	68	8.23±0.06	30	Male	59	1.16±0.02
8	Male	54	2.13±0.06	31	Male	39	<1.00
9	Male	72	1.80±0.48	32	Male	38	3.20±0.18
10	Male	72	ND	33	Male	38	ND
11	Male	62	2.40±0.13	34	Male	32	17.86±0.40
12	Male	65	1.73±0.06	35	Male	36	ND
13	Male	36	ND	36	Male	42	ND
14	Male	54	ND	37	Male	59	ND
15	Male	64	ND	38	Male	60	ND
16	Male	54	ND	39	Male	66	ND
17	Male	46	ND	40	Male	72	ND
18	Male	33	ND	41	Male	47	ND
19	Male	35	ND	42	Male	47	ND
20	Male	30	ND	43	Male	47	ND
21	Male	60	1.33±0.03	44	Male	63	ND
22	Male	52	ND	45	Male	51	2.76±0.16
23	Male	80	1.50±0.04	46	Male	43	ND
24	Male	41	ND	74	Female	64	8.13±0.77
25	Male	41	ND	75	Female	40	ND
26	Male	43	ND	76	Female	60	ND
27	Female	48	ND	77	Female	52	ND
28	Female	50	ND	78	Female	38	ND
29	Female	52	ND	79	Female	60	1.53±0.16
30	Female	58	2.60±0.01	80	Female	50	ND
31	Female	70	ND	81	Female	72	ND
32	Female	70	ND	82	Female	52	ND
33	Female	42	ND	83	Female	42	ND
34	Female	52	<1.00	84	Female	44	ND
35	Female	60	<1.00	85	Female	48	ND
36	Female	50	<1.00	86	Female	56	ND
37	Female	46	ND	87	Female	44	4.13±0.23
38	Female	64	ND	88	Female	66	ND
39	Female	45	ND	89	Female	64	ND
40	Female	49	ND	90	Female	38	7.13±0.19
41	Female	32	ND	91	Female	68	ND
42	Female	37	ND	92	Female	70	ND
43	Female	44	ND	93	Female	32	ND
44	Female	62	ND	94	Female	32	ND
45	Female	54	ND	95	Female	78	ND
46	Female	52	ND	96	Female	34	ND
47	Female	76	ND	97	Female	40	ND
48	Female	56	ND	98	Female	36	ND
49	Female	34	ND	99	Female	38	ND
50	Female	64	ND	100	Female	45	ND

[1] The sample with estimated furan levels below 0.3 ppb.

form, since it readily reacts with protein and DNA nucleophiles (26,31,32). Because *cis*-2-butene-1,4-dial was not detected, it is especially important to use sensitive methods when analyzing for furan levels. In the future, investigations will be aimed at examining other matrices, such as hepatocytes, in order to verify if different matrices can influence furan detection.

In conclusion, this study was performed to establish an analytical method for determining furan levels in human blood by calculating calibration curves, LOQ, and furan recovery rate; the LOQ and recovery rate were 1.0 ppb and 104.1±6.12%, respectively. Furan was detected in the blood samples of 21 individuals (13 males, 8 females), and ranged from 0 to 17.86 ppb; it was not detected in 79 blood samples (36 males, 43 females). Thus, a measurable amount of furan was detected in the samples, and the maximum level was 17.86 ppb. According to the results of this study, the tested analytical method would be robust and accurate in determining human blood levels of furan. The data presented in this study would be helpful in the risk assessment process by describing a method to quantify a potential biomarker of internal dose.

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