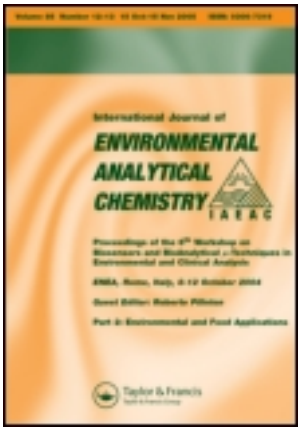

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

Determination of furfural in beers, vinegars and infant formulas by solid-phase microextraction and gas chromatography/mass spectrometry

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Available online: 14 Dec 2011

To cite this article: Shih-Wei Tsai & Kuo-Yuan Kao (2012): Determination of furfural in beers, vinegars and infant formulas by solid-phase microextraction and gas chromatography/mass spectrometry, International Journal of Environmental Analytical Chemistry, 92:1, 76-84

To link to this article: http://dx.doi.org/10.1080/03067319.2010.496050

Intern. J. Environ. Anal. Chem.
Vol. 92, No. 1, 15 January 2012, 76–84

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(*Received 28 January 2010; final version received 12 May 2010*)

The solid-phase microextraction (SPME) technique with on-fibre derivatisation was evaluated for the analysis of furfural in infant formulas, beers, and vinegars. The poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fibre was used and O-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) was first loaded onto the fibre. Food sample of 2 mL was then placed in a 4 mL PTFE-capped glass vial. Headspace extraction by the SPME fibre was performed at 80°C for 20 min under 1100 rpm magnetic stirring with the addition of 40% sodium chloride. Afterwards, the SPME fibre was directly desorbed at the injection port of a gas chromatography/mass spectrometer (GC/MS), followed by the analysis of derivatives formed on-fibre. To avoid matrix interferences, standard addition method was performed. The adsorption-time profiles were examined. The precision, recovery and method detection limits (MDLs) were evaluated with spiked food samples. The relative standard deviations from different spiked samples were all less than 5% and the recoveries were $100 \pm 5\%$. With 2 mL of food sample, MDLs were in the range of $3.09 \sim 14.05\,\mu g\,L^{-1}$. Compared with other techniques, the study shown here provided a simple, fast and reliable method for the analysis of furfural in food samples.

Keywords: solid-phase microextraction; O-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride; furfural; gas chromatography; food samples

1. Introduction

It is accepted that a high proportion of human cancers are due to the exposures of environmental chemicals [1], while human diet was reported to contain a variety of naturally occurring mutagens and carcinogens [2]. For example, furfural is recognised as a dietary mutagen and is present in various foods and beverages [3]. Therefore, certain foods in some countries have been related to the incidence of certain types of cancers in the populations [2].

Furfural is also a widely used industrial chemical. The US Environmental Protection Agency (EPA) estimated the range of US production was 1.12×10^7 to 4.57×10^7 kg in 1994 [4]. Besides fungicide and herbicide [5], furfural is often used as a selective solvent in the production of lubricating oils, as a reactive wetting agent in the production of

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ISSN 0306–7319 print/ISSN 1029–0397 online

http://dx.doi.org/10.1080/03067319.2010.496050
http://www.tandfonline.com

refractory components, and for the resin blinder system in the production of abrasive wheels [6].

Furfural in food items arises through thermal degradation of carbohydrates and ascorbate [7]. As mentioned above, furfural has been identified from various foods and beverages, including beef, soy sauce, roasted nuts, fried bacon, nectarines, baked potatoes, clove oil, preserved mangoes, rum, roasted coffee and blue cheese [8]. Since furfural is known as a dietary mutagen, its presence in foods has raised toxicological concerns. However, the levels of furfural in foods and beverages are usually low. Therefore it has always been a challenge to develop an analytical method that would enable routine analysis.

Several analytical methods have been developed to determine aldehydes, including furfural, in food products. For example, liquid-liquid extraction [9], distillation [10] and sorbent extraction [11] have all been used for the sample preparations. However, these methods are rather complicated and not highly selective [12].

To overcome the disadvantages, such as the time-consuming and solvent-using problems, Pawliszyn has developed an extraction technique called solid-phase micro-extraction (SPME) [13,14]. SPME presents many advantages over conventional analytical methods by combining sampling, preconcentration and direct transfer of the analytes into a standard gas chromatograph (GC) system [15]. There have been many applications of SPME in the environmental field. For example, aldehydes derivatised with PFBHA to form oximes in solutions followed by the extraction with SPME from liquid or headspace and analysed by GC/ECD was reported [16].

For the determination, derivatisations prior to their detection by a spectroscopic or chromatographic technique are widely performed for the low-molecular-mass aldehydes [17]. Various derivatisation techniques including direct derivatisation in sample matrix, derivatisation in GC injector port and derivatisation in SPME fibre coating can also be implemented combined with SPME [13]. Among them, the on-fibre derivatisation technique will not only increase the sample stability but will also allow high efficiencies and can be used in remote field applications [13].

The technique of on-fibre derivatisation where oximes formed after O-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) reacted with aldehydes has been reported elsewhere for the determination of aldehydes in water [18]. Compared with the other PFBHA-SPME method for the analysis of aldehydes in water where oximes were formed in solutions and vaporised to headspace by magnetic stirring [16], the on-fibre derivatisation provides an analytical method with easy operation and better sensitivity [18].

In this research, the SPME procedure with headspace extraction was performed to reduce background adsorption and matrix effects from food samples, including infant formulas, beers and vinegars. PFBHA was used as the derivatising agent to enhance stability. Effects of experimental parameters on both headspace SPME and on-fibre derivatisation were investigated as well. In addition, standard addition was performed to avoid matrix interferences.

2. Experimental

2.1 *Materials*

Furfural, O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA), n-hexane, and methanol were purchased from Sigma-Aldrich (Milwaukee, WI, USA).

Helium for GC/MS was 99.999% purity from Sanfu Co., Taiwan. All solid-phase microextraction (SPME) fibres, holders and molecular sieve were from Supelco (St. Louis, MO, USA). The food samples, including infant formulas, beers and vinegars, were purchased from major supermarkets in Taichung, Taiwan.

2.2 *Instrumentation*

All analyses were performed on a Perkin Elmer Autosystem XL Chromatograph equipped with a $30\,m \times 0.25\,mm$ I.D. $0.25\,\mu m$ film DB-WAX chemically bonded fused-silica capillary column (J&W Scientific, Folsom, CA, USA) and a Perkin Elmer Turbo Mass, mass spectrometer. The carrier gas was helium with flow rate of $1.0 \pm 0.1\,mL\,min^{-1}$ in the 1 : 10 split mode. The temperature for the injector was 250°C. The column temperature program was: 120°C to 200°C at 10°C/minute, and hold for 3 minutes. The temperature of mass spectrometer was 250°C.

2.3 *Loading SPME fibres with PFBHA*

Poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) SPME fibre (65μm) was selected because it adsorbed PFBHA with greater reproducibility [19]. A solution of PFBHA ($17\,mg\,mL^{-1}$) in aldehydes-free water was placed in 4 mL PTFE-capped vials with a 1 cm stir bar [19]. The magnetic stirrer used was MIRAK 7×7 stir plate from Barnstead International (Dubuque, Iowa, USA) which also allowed temperature control. The solution was stirred at 1100 rpm. Then the PDMS/DVB SPME fibre (65 μm) was placed in the headspace of the solution above the center of the solution. To obtain the adsorption-time profile, the SPME fibres were exposed to the vapours of the aqueous solutions for 0.5, 1, 1.5, 2, 5, 10, 20 and 30 min, respectively. Chromatographic peak areas and calibration curves were used for adsorbed PFBHA quantification. To ensure the desorption was complete when the SPME needle was inserted into the heated GC injector, different desorption times were tested to examine the desorption efficiencies. For successive analysis of samples, the SPME fibre was always first heated in the GC injector and a blank run was performed before the loading of PFBHA to make sure the fibre was clean as well as to avoid the carry-over effects.

2.4 *Derivatisation and SPME procedures for quantification*

Headspace extraction was used in this research to avoid possible contamination and damage to the fibre that might occur through direct liquid contact [20]. Since different matrix may significantly affect the distribution of furfural between sample materials and gas phase, the calibrations in this research were performed by standard addition method to avoid matrix interferences from food samples.

In a 4 mL PTFE-capped vial with 1 cm stir bar, 2 mL of food sample was first placed in, followed by the addition of 20 μL solution of furfural with known concentration. For the quantification, six vials of the same food sample were prepared, and the concentrations of furfural solutions spiked ranged from $1.16 \sim 928\,mg\,L^{-1}$, respectively. The solutions were then stirred at 1100 rpm for at least 5 min before further procedures were performed to allow the equilibrium of analytes between the aqueous phase and the headspace phase. After loading with PFBHA, the SPME fibre was inserted in the headspace of the solutions.

Different periods of time for extraction were performed to obtain the adsorption-time profiles.

To determine the precision and accuracy of current technique, spiked samples (final concentration of furfural equal 0.23 mg L^{-1}) of infant formulas, beers and vinegars were analysed ten times based on the processes mentioned above, respectively. The relative standard deviation (R.S.D.) and accuracy for each food sample were then calculated. Besides, spiked samples with concentration of furfural equal 0.023 mg L^{-1} were also analysed seven times to determine the method detection limits (MDLs) based on the following procedures [21]:

$$\mathrm{MDL} = \mathrm{St}_{(n-1,1\text{-alpha}=0.99)}$$

where:

MDL = the method detection limit
$t_{(n-1,\ 1\text{-alpha}=0.99)}$ = Student's t value for the 99% confidence level with $n-1$ degrees of freedom
n = number of replicates
S = standard deviation of replicate analyses
for $n=7$ and alpha = 0.01, $t_{(n-1,\ 1\text{-alpha}=0.99)}=3.143$.

3. Results and discussion

To upload PFBHA onto PDMS/DVB fibre, a solution of PFBHA in aldehydes-free water was placed in a 4 mL PTFE-capped vial with a 1 cm stir bar and the solution was stirred at 1100 rpm [22]. Loading time of 2 min was used in current study based on previous experience [18]. More PFBHA can be loaded on the fibre if the time for extraction is increased. The condition for thermal desorption of the SPME fibre was also determined. At a temperature of 250°C, the desorption efficiency was found to be 99.96% when the desorption time was 2 min.

Figure 1 shows the SPME adsorption-time profiles from the on-fibre derivatisations with PFBHA of furfural spiked in food samples. As the time for extraction increased, the amounts of oximes formed increased as well. For vinegar, it was observed that 80% of the equilibrium reached when the adsorption time was around 15 min. As for beer and infant formula, over 90% of the equilibrium can be observed when the time was around 15 min, respectively. The amounts of furfural spiked for vinegar, beer and infant formula were the same. However, as shown in Figure 1, it seems that the furfural in vinegar is easier to be vaporised than in beer and in infant formula. Complex matrix of the infant formula might explain why the response was relatively low.

Figure 2 shows a typical GC/MS chromatogram of spiked vinegar sample from the SPME direct injection with selective ion monitoring (SIM) utilising m/z 181 [23]. It was observed that there were syn and anti isomers of the oxime because furfural was an asymmertrical carbonyl compound.

When the salt concentration in the solutions is increased, the amount extracted is increased frequently because the fibre/matrix distribution constant increases [13]. However, a decrease in the amount extracted is sometimes observed when analytes are in dissociated form [13]. Some researchers also found that the addition of salt might have no significant effect on the amount extracted. For example, the addition of 10% NaCl had no significant effects on the extractability of the PFBHA derivatives [16]. In current study,

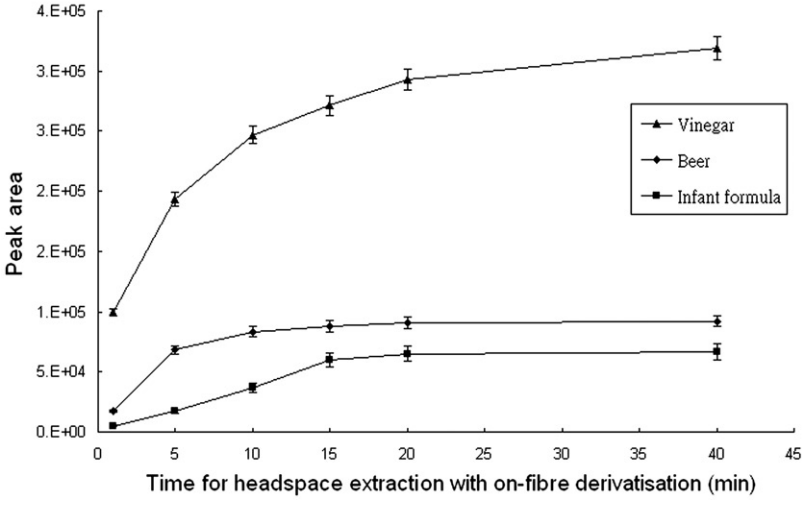

Figure 1. Adsorption-time profiles for furfural in food samples using headspace SPME with on-fibre derivatisation. Sample volume was 2 mL and spiking level of furfural was 27.84 mg L^{-1}.

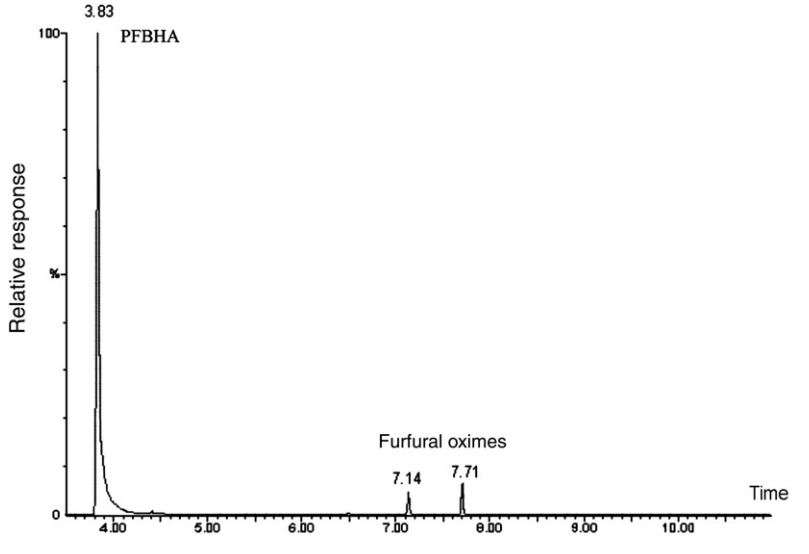

Figure 2. Typical GC/MS chromatograms of spiked vinegar sample. Sample volume was 2 mL and spiking level of furfural was 0.23 mg L^{-1}.

the effects of salt additions were also investigated and Figure 3 shows the results (the spiked amount was 27.84 mg L^{-1} of food sample). For vinegar, the amounts of oximes formed on-fibre increased as the concentration of NaCl increased, apparently. As for the samples of beer and infant formula, it seems that the additions of NaCl also had positive effects on the amounts of oximes formed on-fibre.

Besides the effect of NaCl addition, the influences of different extraction temperatures were investigated as well and Figure 4 shows the results. The data from vinegar and beer

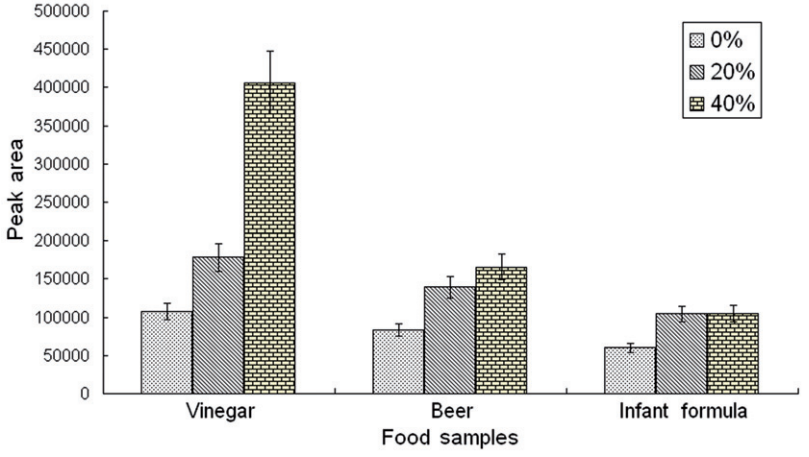

Figure 3. Effects of salt (NaCl) addition of spiked food samples. Sample volume was 2 mL and spiking level of furfural was 27.84 mg L^{-1}.

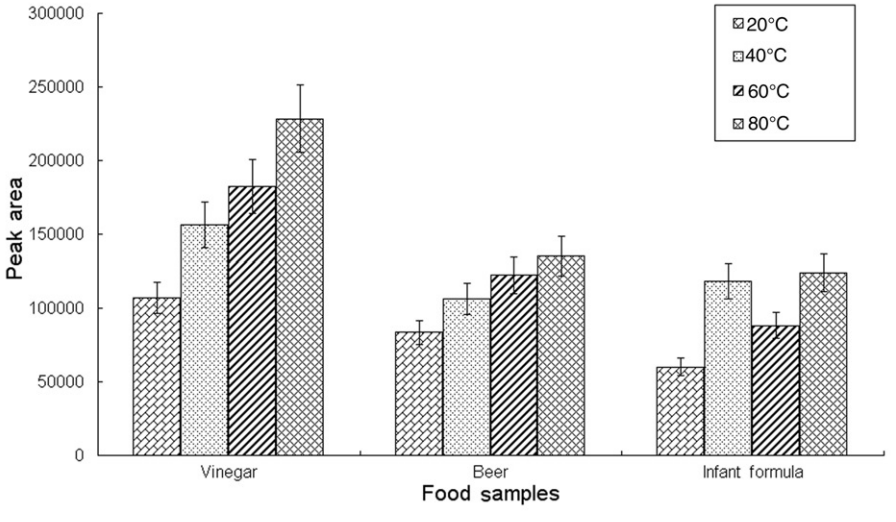

Figure 4. Effects of headspace extraction temperatures of spiked food samples. Sample volume was 2 mL and spiking level of furfural was 27.84 mg L^{-1}.

clearly demonstrated the dependence of extraction temperatures. However, the reason why infant formula showed a different profile was not clear. For further validations of the method, 40% of NaCl addition and headspace extraction at 80°C were performed to reach higher sensitivities for all the food samples tested in this research.

Various kinds of food samples, including beer, infant formula and vinegar, were spiked with known amounts of furfural to determine the precision, accuracy and MDLs of the method developed (Table 1 shows the results). It was found the R.S.D.s from the current method were all satisfactory according to Horwitz [24]. Besides SPME methods,

Table 1. Comparisons of different methods for the analysis of furfural in food samples.

Food samples	Sample pretreatments	Analytical methods	Linear range	MDLs	Accuracy & precision	Ref.
Beer	SPME swith headspace extraction	GC/MS	$0.1 \sim 50\,mg\,L^{-1}$	–	99(5.1)[b]	[12]
Milk-based formulae	mixed with trichloroacetic acid + centrifugation	HPLC-diode array detection	$0.05 \sim 2\,mg\,L^{-1}$	$12\,\mu g\,L^{-1}$	96.24(4.28)	[25]
Vinegar	SPME with headspace extraction	GC/MS using isotope dilution	$0.12 \sim 16\,mg\,L^{-1}$	$15\,\mu g\,L^{-1}$	94.0(14.9)	[26]
Beer Infant formula Vinegar	SPME with headspace extraction	On-fibre derivatisaton with PFBHA; analysed by GC/MS	Beer: $0.023 \sim 46.40\,mg\,L^{-1}$ Infant formula: $0.023 \sim 46.40\,mg\,L^{-1}$ Vinegar: $0.023 \sim 27.87\,mg\,L^{-1}$	Beer[a]: $8.24\,\mu g\,L^{-1}$ Infant formula[a]: $14.05\,\mu g\,L^{-1}$ Vinegar[a]: $3.09\,\mu g\,L^{-1}$	Beer[c]: 95.55(4.74) Infant formula[c]: 95.96(1.96) Vinegar[c]: 96.74(1.38)	Current study

[a] $n = 7$, sample volume = 2 mL; spiked concentration = $0.023\,mg\,L^{-1}$.
[b] Accuracy = 99%, relative standard deviation = 5.1%.
[c] $n = 10$, sample volume = 2 mL; spiked concentration = $0.23\,mg\,L^{-1}$.

traditional technique such as liquid extraction has also been applied in the literature for the determination of furfural from food samples [25]. However, liquid extraction requires solvents and cumbersome procedures. For example, 10 mL of 0.2 N oxalic acid was mixed with 2 g of formula power and heated in a water-bath at 100°C for 25 min, before a further 15 mL of 40% (w/v) trichloroacetic acid was used for the extraction (the time needed for the whole procedure was 80 min, at least) [25].

Wide concentration ranges of furfural in foods have been reported elsewhere [12,25–26]. For example, $0.31 \sim 14.19\,\mathrm{mg\,L^{-1}}$ of furfural were determined from different vinegar samples. Regarding the detection sensitivities for various matrix, as shown in Table 1, the current method proposed MDLs were in the range of $3.09 \sim 14.05\,\mu\mathrm{g\,L^{-1}}$ with only 2 mL of sample used.

Compared with other SPME studies, the current method also produced lower linear range in measuring furfural in food samples. For example, it was reported that the range for quantitation was $0.1 \sim 50\,\mathrm{mg\,L^{-1}}$ for furfural in beer by the method of SPME with headspace extraction [12], while it was lowered to $0.023\,\mathrm{mg\,L^{-1}}$ in this research by the on-fibre derivatisation with PFBHA. For the vinegar sample, it was reported that the linear range was $0.12 \sim 16\,\mathrm{mg\,L^{-1}}$ by using GC/MS with isotope dilution [16], while the lower quantitative concentration also reached $0.023\,\mathrm{mg\,L^{-1}}$ in this research for vinegar. The possible reason for the results was the unique procedure in this technique which involved the reaction with PFBHA. With on-fibre derivatisation, the improvement of quality and sensitivity for separations were observed.

Compared with other PFBHA-SPME method for the analysis of aldehydes in water where oximes were formed in solutions and vaporised to headspace by magnetic stirring [16], furfural in food samples were stirred to headspace and reacted with PFBHA on-fibre in current research. It was obvious that vaporising furfural was easier than oximes because the molecular masses were far different (e.g. 96 g/mole for furfural while 291 g/mole for furfural-PFBHA oxime). This might explain why a 15 min extraction time could be used here to yield over 80% of extraction efficiencies for all the food samples tested in this study, while other researchers had to use a 30 min extraction time for water samples [16].

4. Conclusions

This research focused on the validation of analytical method for furfural in food samples based on the technique of SPME with on-fibre derivatisation. The poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) SPME fibre (65 μm) was used and a sample preparation procedure was established. Food samples of 2 mL were first placed in a 4 mL PTFE-capped glass vial. Headspace extraction of furfural in the food sample was then performed under 80°C for 20 min with 1100 rpm magnetic stirring and the addition of 40% sodium chloride. Afterwards, GC/MS was used for the analysis of derivatives formed on-fibre.

Compared with traditional methods, this method reduced and simplified the experimental procedure and omitted the use of organic solvents. The on-fibre derivatisation with PFBHA in this research provided an efficient analytical tool with acceptable linear relationship, MDLs, precision and accuracy. The time saving procedure also makes the proposed method suitable for routine analysis of food samples.

Acknowledgement

This study was supported by grant from the National Science Council, Executive Yuan, Taiwan (NSC 93-2320-B-039-012).

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