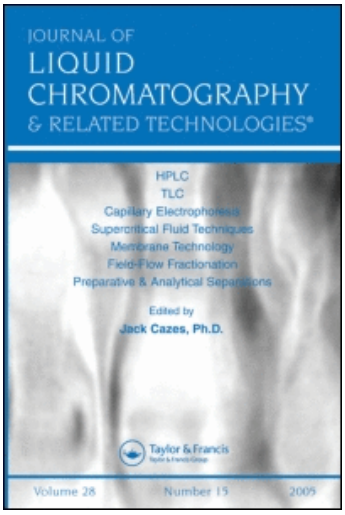

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HPLC Determination of Furfural after Preliminary Extraction to Aqueous Phase

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Abstract: Furfural contents in several carbohydrate rich foods were measured by HPLC in 8 minutes. A wide linear dynamic range (0.07–40 $\mu g\ mL^{-1}$), detection limit of 0.07 $\mu g\ mL^{-1}$, quantitaion limit of 0.21 $\mu g\ mL^{-1}$, and sensitivity of 77.17 $mAU \cdot (\mu g\ mL^{-1})^{-1}$ were obtained. Deterioration in carbohydrate rich foods was evaluated by furfural analysis before and after storage and an increase in furfural was observed in all samples due to storage. A simple, rapid, and economical liquid liquid extraction procedure was proposed for extraction of furfural from oily samples. Recovery of 88.43% was obtained. Extracted furfural in aqueous phase was determined by the HPLC method. HPLC also was applied to the determination of furfural in waste water of the Behran motor oil company.

Keywords: Furfural, HPLC-UV, Carbohydrate rich foods, Oil, Waste water

INTRODUCTION

The major components of many foods, such as fruit juices and honey are sugars. Sugars decompose into furfural compounds by two ways: via Amadori compounds from Millard reaction by ennolization in acidic conditions, or through lactose isomerization.[1] Furfural compounds are

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common products of these reactions.[2] Furfural compounds accumulate in sugar rich foods during treatment or storage.[3–5]

The toxicological relevance of furfural compounds is not clear as in vitro studies on genotoxicity and mutagenicity have given controversial results.[6–8] However, furfural is considered an undesirable compound. Furfural compounds can be used as an indicator of food damage due to treatment or storage for a wide range of carbohydrate rich foods such as processed fruits,[4,5,9] honey,[10,11] and milk.[12]

Hydrophilic organic compounds, such as furfural are miscible with water as well as several organic solvents. Determination of these toxic compounds is important for estimating their effect on human health.[13] Refineries use furfural compounds for elimination of aromatic compounds from motor oils and, thus, furfural can be introduced into environmental waters from the waste water of refineries.

Different analytical methods were developed in the last years to determine furfural compounds in environmental and food samples. These methods initially were spectrophotometric measurements.[14] Because these methods are time consuming and not specific, HPLC and gas chromatography were used as rapid and selective methods for determination of these compounds in environmental and food samples.[13,15–17] Kawata et al.[13] tried to determine hydrophilic organic compounds, such as furfural in environmental water, by use of gas chromatography-mass spectrometry. Servin et al.[17] applied HPLC for determination of furfural compounds milk based formulae with detection limit of 32.07 $\mu g/100$ g and 15 minute analysis time.

For the first time in the present work a simple liquid liquid extraction procedure was developed for pulling out furfural from non-aqueous samples. The furfural was determined in the extracted phase and also in food and waste waters by an HPLC method with a good detection limit and analysis time. It was proposed that the furfural analysis is useful for evaluation of deterioration in food samples.

EXPERIMENTAL

Reagents and Materials

Furfural was purchased from Fluka (Buchs, Switzerland). Methanol and acetonitrile (HPLC grade), hexane, and sodium disulfite were obtained from Merck (Darmstadt, Germany).

Apparatus

Agilent technologies 1100 series chromatographic system consisting of quaternary pump G1311A (Agilent technologies, Waldbronn, Germany), multiple wavelength detector G1365B (Agilent technologies, Waldbronn,

Germany), manual injector (Rheodyne, Rohnert Park, CA, USA) equipped with 20 μL injection loop and degasser (Agilent technologies, Tokyo, Japan) were used. Data were collected and analyzed using the Chem. Station for LC 3D software package. A Zorbax Eclipse XDB-C8 column 150 × 4.6 mm, 5 μm particle size (Agilent technologies, Santa Clara, CA, USA) was used.

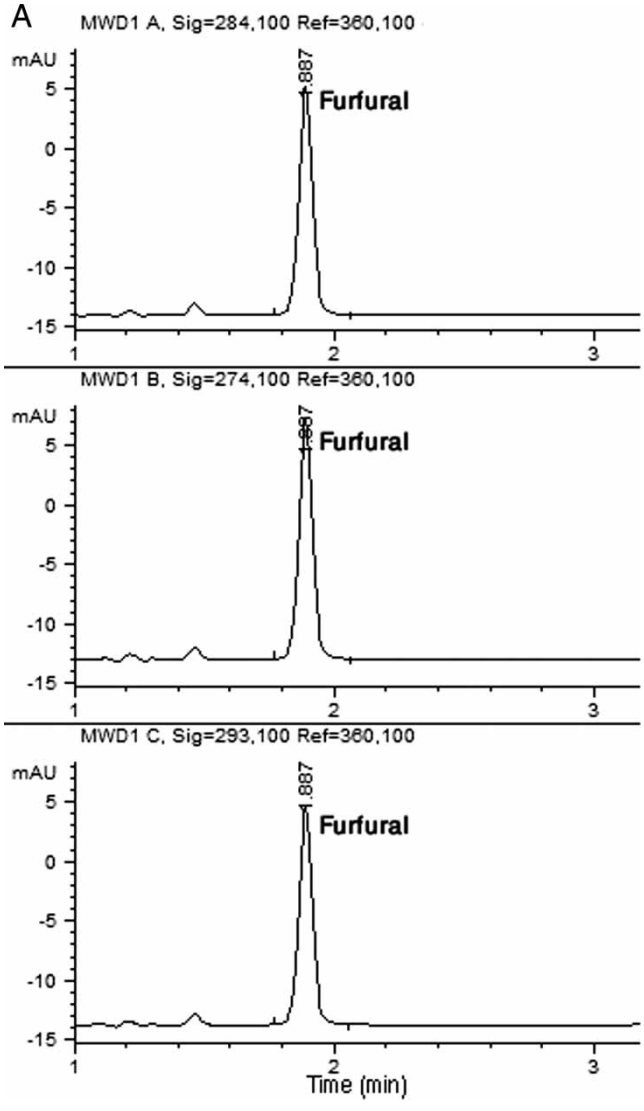
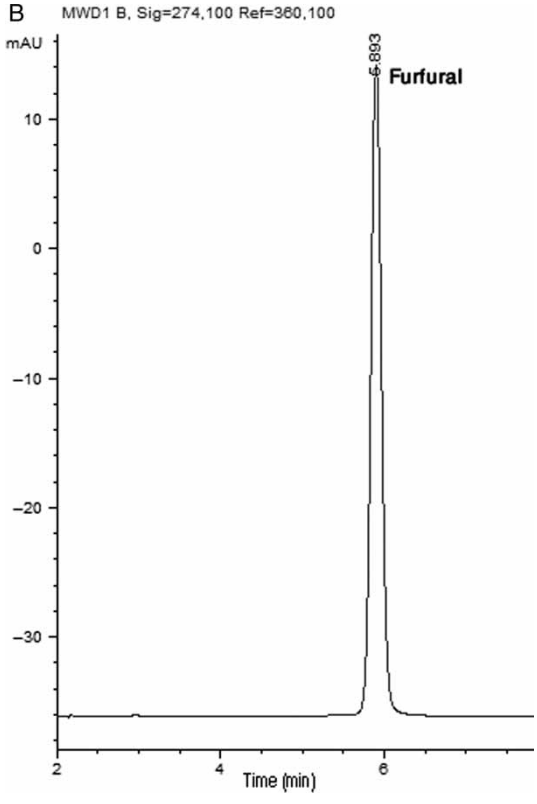


Figure 1. Chromatogram of 10 μg mL^{-1} furfural standard solution in optimum condition: A. At first injection; B. After approximately 200 injection. (*Continued*)



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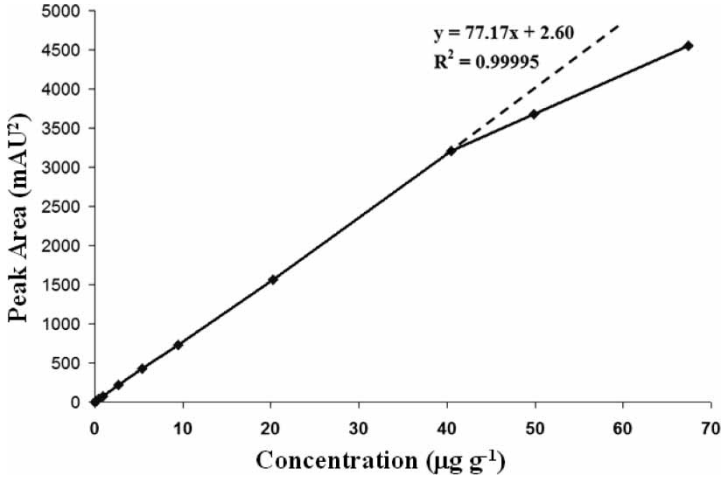

Figure 2. Calibration curve for furfural determination using HPLC.

Table 1. HPLC analysis on spiked and non-spiked oil samples

	Furfural	
	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)
Oil sample[a]	—	0.72 ± 0.04
Oil sample[b]	0.75	1.30 ± 0.04

Values are expressed as calculated value from regression equation $\pm$ standard deviation.

[a]Refined oil sample without spike.

[b]Refined oil sample with spike.

All samples were filtered in two steps: first by filter paper No. 44 (Whatman, Brentford, UK) and then using cellulose membrane syringe filters (Agilent technologies, Waldbronn, Germany) with 0.45 μm pore size and 13 mm diameter.

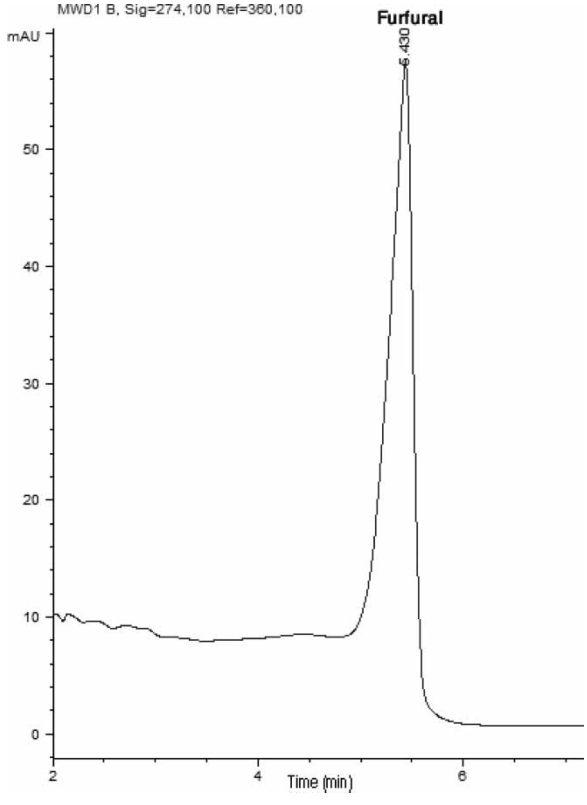

Figure 3. Chromatogram of extracted solution of oil sample.

Sample Preparation

A stock solution of furfural (1000 μg mL^{-1}) was prepared by weighting a 0.1 g furfural, dissolved in water and diluted to 100 mL in a volumetric flask. All working standard solutions were prepared from this stock solution by dilution. All food samples were miscible with water, thus, an appropriate amount of sample was dissolved in ultra-pure water and then filtered. Oily sample preparations were based on liquid-liquid extraction. Approximately 2 g of oily sample was dissolved in 25 mL hexane and was extracted with two separate 40 mL portions of sodium disulfite aqueous solution (10% w/v) by using a 250 mL separatory funnel and shaking vigorously for 2 minutes. The extracts were combined and diluted in a 100 mL volumetric flask.

RESULTS AND DISCUSSION

Chromatographic Method

Characterization of proposed HPLC method for furfural analysis was performed by injection of a 10 μg mL^{-1} furfural standard solution into the

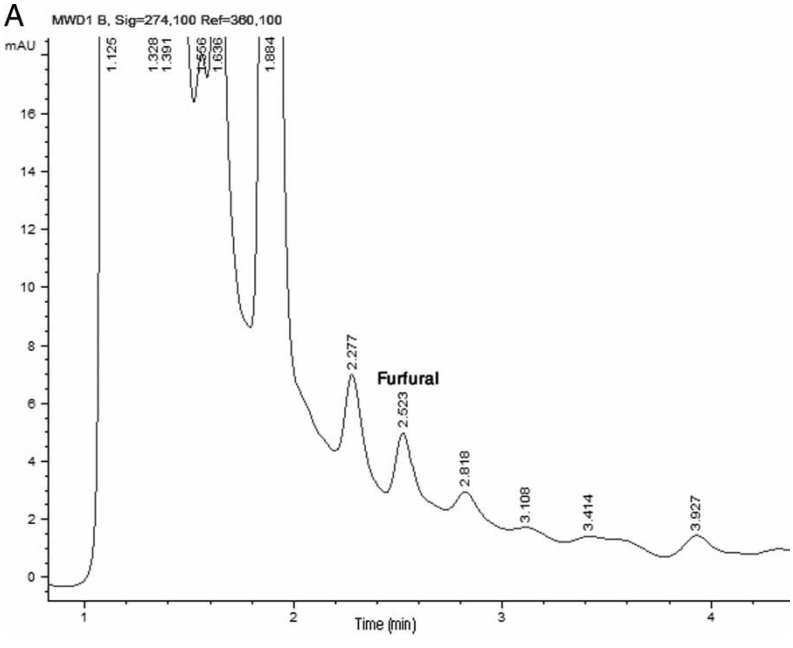

Figure 4. Chromatogram of grape juice: A. Without spike; B. After spike of 9 μg mL^{-1} furfural standard solution.

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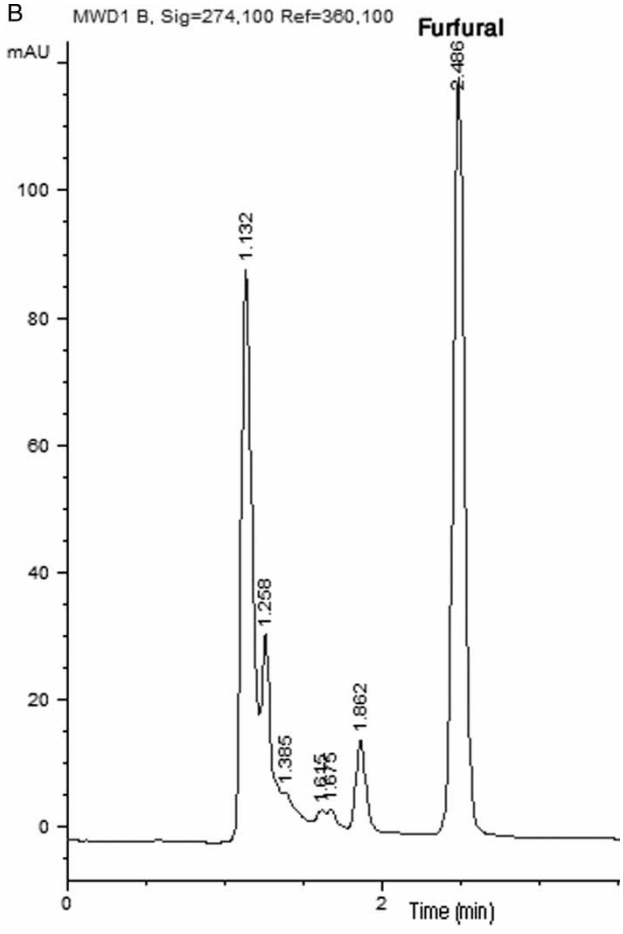

Figure 4. Continued.

HPLC system. The mobile phase, with slight variations of water-methanol-acetonitrile, was examined and the detector was simultaneously set at 274 nm, 284 nm, and 293 nm (Figure 1A). It was observed that a 90:5:5 ratio at 274 nm gave the best results. The retention time was changed from 1.887 min to 5.893 min after approximately 200 injections (Compare Figure 1A and 1B). The retention time increased without decreasing efficiency of the column.

Statistical Parameters of Chromatographic Method

The linear dynamic range, sensitivity, and detection limit of the method were studied using furfural standards. Figure 2 shows the plot of peak area versus

concentration of furfural. The calibration graph shows that the response is linear over the range 0.07 40 $\mu g\ mL^{-1}$; the calibration sensitivity is 77.17 $mAU\cdot(\mu g\ mL^{-1})^{-1}$. The detection limit of 0.07 $\mu g\ mL^{-1}$ was calculated according to 3.3 s/S, where s is the standard deviation of the y intercept of the regression line and S is the calibration sensitivity.[18] The quantitation limit of 0.21 $\mu g\ mL^{-1}$ was calculated (10 s/S).

Recovery

Recovery of furfural from a 2 g oil sample was investigated by adding 1.5 mg of the standard compound to the oil sample. The mixture was dissolved in

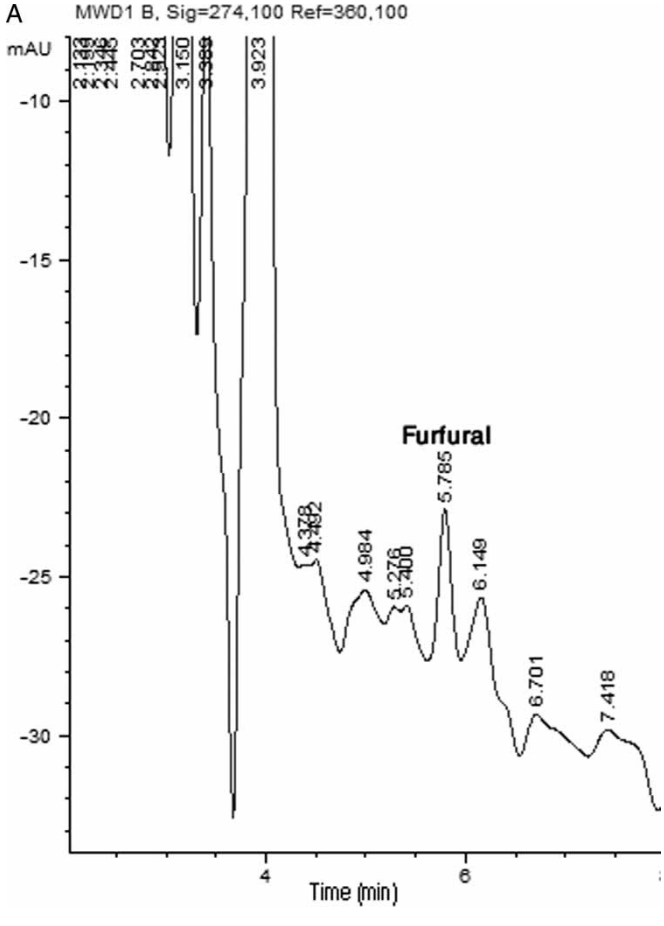

Figure 5. Chromatogram of mixture of vinegar and syrup: A. Without spike; B. After spike of 9 $\mu g\ mL^{-1}$ furfural standard solution.

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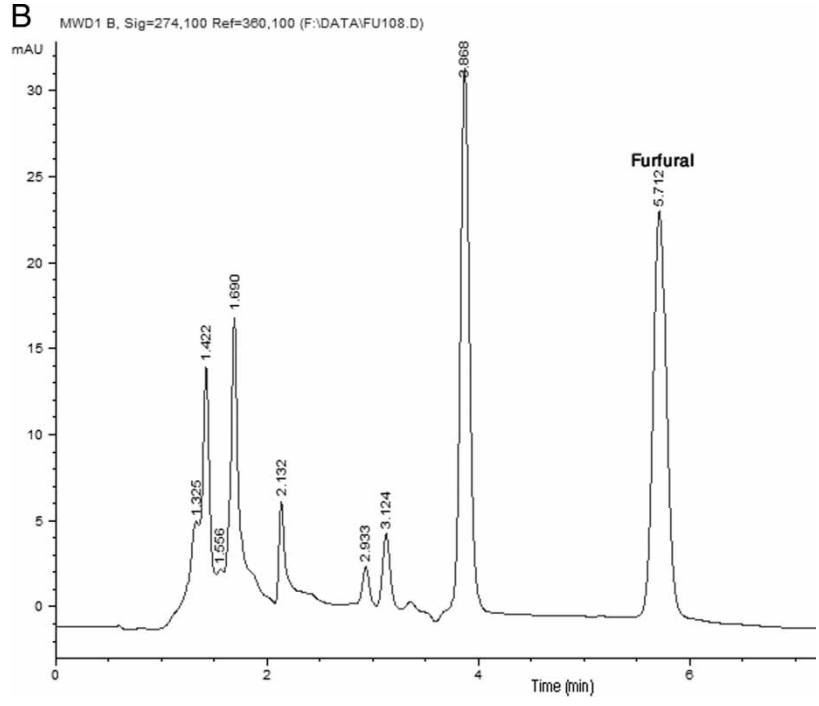

Figure 5. Continued.

25 mL hexane and extracted according to the sample preparation procedure. The extraction procedure was also applied to a solution of 2 g of oil sample in 25 mL hexane (without spike). The two extract solutions were analyzed by HPLC and results are shown in Table 1. Figure 3 shows the chromatogram of the spiked oil sample after extraction. According to these results recovery of 88.43% was calculated.

Table 2. Results of furfural analysis on foods

Sample	Furfural at production date ($\mu g\ g^{-1}$)	Furfural after 6 months ($\mu g\ g^{-1}$)
Grape juice	0.35 ± 0.04	0.79 ± 0.04
Honey	N.D.[a]	N.D.[a]
Mixture of vinegar and syrup	N.D.[a]	4.39 ± 0.04

Values are expressed as calculated value from regression equation $\pm$ standard deviation.

[a]Not detected.

Table 3. Furfural in waste water of Behran motor oil company

	Sample 1^a ($\mu g\ g^{-1}$)	Sample 2^b ($\mu g\ g^{-1}$)
Furfural	0.26 ± 0.04	0.08 ± 0.04

Values are expressed as calculated value from regression equation $\pm$ standard deviation.

[a]Before filtration.

[b]After filtration.

Study on Deterioration of Foods by Furfural Analysis

Several food samples containing grape juice (Rijav Co., Tehran, Iran), honey (Ardabil, Iran), and a mixture of vinegar and syrup (home made) were analyzed at the production date and after 6 months, using the HPLC method (see Figure 4 and 5). Results were shown in Table 2. It appeared that storage of these carbohydrate rich foods can increase the furfural concentration, so furfural analysis is useful for evaluating deterioration in these

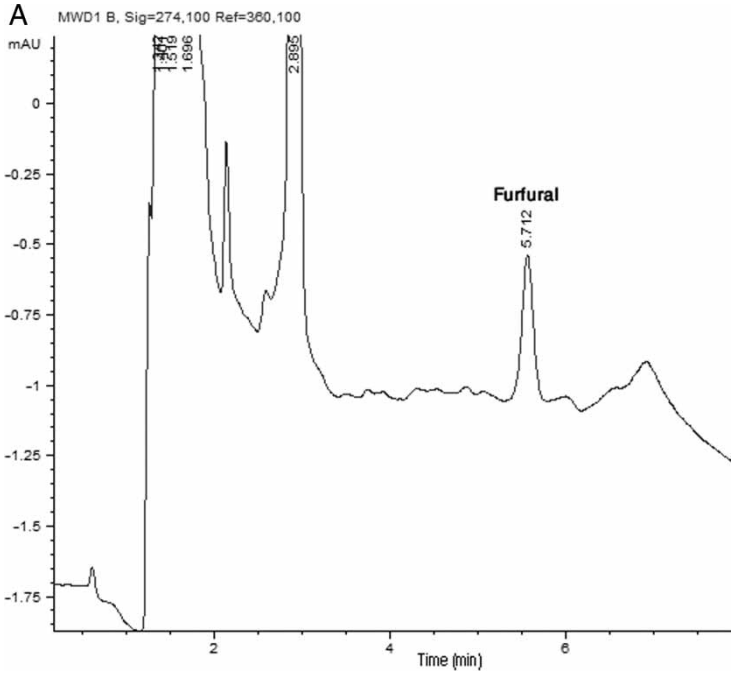

Figure 6. Chromatogram of Behran Company waste water: A. Before filtration; B. After filtration.

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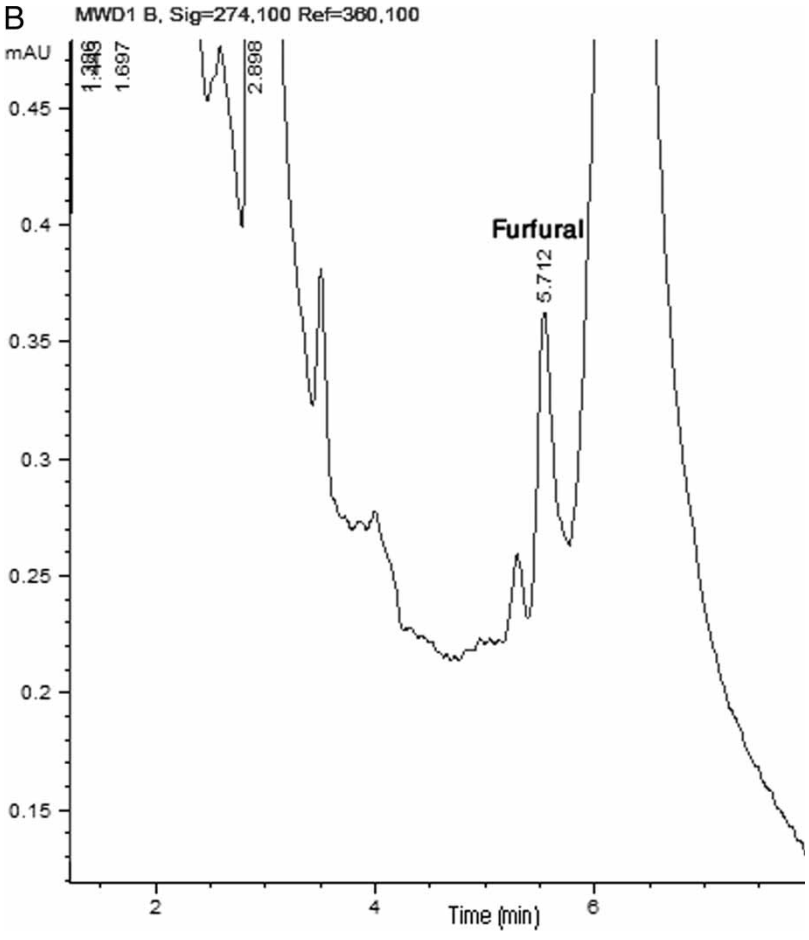

Figure 6. Continued.

types of food samples. Also, the furfural formation in food samples increase with acidity, thus, in the mixture of syrup and vinegar the rate of furfural formation is high.

Determination of Furfural in Waste Waters

Two samples were taken from waste water of the Behran motor oil company (Tehran, Iran) before and after filtration. Table 3, shows results of HPLC analysis (see Figure 6) on these samples. Although filtration removes 69.23% of furfural from waste water, furfural remaining in waste water at 0.08 $\mu g/g$ level can be introduced into the environment.

CONCLUSIONS

A new HPLC condition was proposed as a simple and fast method with good linear dynamic range, detection limit, and sensitivity for determination of furfural in several carbohydrate rich foods. It was shown that furfural analysis is useful for the evaluation of deterioration in food samples.

Our study demonstrates the use of sodium disulfite aqueous solution as the extracting medium for furfural from oily samples with 88.43% recovery. Furfural was analyzed in extracted aqueous samples by the HPLC method. Waste water of the Behran Oil Company before and after filtration was analyzed by this method.

The results obtained point to the need for strict control of expiration dates of carbohydrate rich foods in order to ensure the greatest nutritional quality of products of this kind. On the other hand, from the human health point of view, there is a need to improve controls on the waste water of refineries.

REFERENCES

1. Brands, C.M.J.; Boekel, M.A.J.S.V. Reactions of monosaccharides during heating of sugar-casein systems: Building of a reaction network model. J. Agric. Food Chem. **2001**, *49*, 4667–4675.
2. Ameur, L.A.; Trystram, G.; Aragon, I.B. Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method. Food Chem. **2006**, *98*, 790–796.
3. Akkan, A.A.; Ozdemir, Y.; Ekiz, H.L. Derivative spectrophotometric determination of 5-(hydroxymethyl)-2-furaldehyde (HMF) and furfural in locust bean extract. Nahrung Food **2001**, *45*, 43–46.
4. Ibraz, A.; Pagan, J.; Garza, S. Kinetic models of non-enzymatic browning in apple puree. J. Sci. Food Agric. **2000**, *80*, 1162–1168.
5. Mendoza, M.R.; Sanz, M.L.; Olano, A.; Villamiel, M. Formation of hydroxymethylfurfural and furosine during the storage of jams and fruit-based infant foods. Food Chem. **2004**, *85*, 605–609.
6. Cuzzoni, M.T.; Stoppini, G.; Gazzani, G.; Mazza, P. Influence of water activity and reaction temperature of ribose-lysine and glucose-lysine millard systems on mutagenicity, absorbance and content of furfurals. Food Chem. Toxicol. **1998**, *26*, 815–822.
7. Janzowski, C.; Glaab, V.; Samimi, E.; Schlatter, J.; Eisenbrand, G. 5-Hydroxymethylfurfural: Assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. Food Chem. Toxicol. **2000**, *38*, 801–809.
8. Lee, Y.C.; Shlyankevich, M.; Jeong, H.K.; Douglas, J.S.; Surh, Y. Bioactivation of 5-hydroxymethyl-2-furfuraldehyde to an electrophilic and mutagenic allylic sulfuric acid ester. Biochem. Biophys. Res. Commun. **1995**, *209*, 996–1002.
9. Mendoza, M.R.; Olano, A.; Villamiel, M. Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. Food Chem. **2002**, *79*, 513–516.
10. Fallico, B.; Zappala, M.; Arena, E.; Verzera, A. Effects of conditioning on HMF content in unifloral honeys. Food Chem. **2004**, *85*, 305–313.

11. Tosi, E.; Ciappini, M.; Re, E.; Lucero, H. Honey thermal treatment effects on hydroxymethylfurfural content. Food Chem. **2002**, *77*, 71–74.
12. Morales, F.J.; Perez, S.J. Hydroxymethylfurfural determination in infant milk-based formulas by micellar electrokinetic capillary chromatography. Food Chem. **2001**, *72*, 525–531.
13. Kawata, K.; Ibaraki, T.; Tanabe, A.; Yagoh, H.; Shinoda, A.; Suzuki, H.; Yasuhara, A. Gas chromatographic-mass spectrometric determination of hydrophilic compounds in environmental water by solid-phase extraction with activated carbon fiber felt. J. Chromatogr. A **2001**, *911*, 75–83.
14. Tu, D.; Xue, S.; Meng, C.; Mansilla, A.E.; Pena, A.M.D.L.; Lopez, F.S. Simultaneous determination of 2-furfuraldehyde and 5-(hydroxymethyl)-2-furfuraldehyde by derivative spectrophotometry. J. Agric. Food Chem. **1992**, *40*, 1022–1025.
15. Ferrer, E.; Alegria, A.; Farre, R.; Abellan, P.; Romero, F. High performance liquid chromatographic determination of furfural compounds in infant formulas: Changes during heat treatment and storage. J. Chromatogr. A **2002**, *947*, 85–95.
16. Ferrer, E.; Alegria, A.; Farre, R.; Abellan, P.; Romero, F. High performance liquid chromatographic determination of furfural compounds in infant formulas during full shelf-life. Food Chem. **2005**, *89*, 639–645.
17. Servin, J.L.C.; Castellote, A.I.; Sabater, M.C.L. Analysis of potential and free furfural compounds in milk-based formulae by high performance liquid chromatography evolution during storage. J. Chromatogr. A **2005**, *1076*, 133–140.
18. Christian, G.D. *Analytical Chemistry*; John Wiley & Sons: Hoboken, NJ, USA, 2004; pp. 112–113.

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