JOTCS A
Journal of the Turkish Chemical Society Section: A Chemistry
TURKISH CHEMICAL SOCIETY

Determination of 5-hydroxymethylfurfural (5-HMF) in Expired Pharmaceutical Syrups by Using HPLC-DAD Method

Songül Ünüvar*

University of Inonu, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 44280,Malatya, Turkey.

Abstract: The Maillard reaction product 5-hydroxymethylfurfural (5-HMF) is formed under acidic conditions by the dehydration of sugars in carbohydrate-based food and pharmaceutical products during heating and storage. As pharmaceutical syrup formulations contain sugar and are stored under room temperature, they provide favorable conditions for the formation of 5-HMF. The long-term storage of syrup bottles after their cap has been opened and the unintentional use of expired syrups can lead to the formation of undesirable products such as 5-HMF in medications. Although legal limits have been established for 5-HMF content in pharmaceutical preparations, these levels may exceed those limits in hot climates or under inappropriate storage conditions. The present study detects and measures 5-HMF levels in expired pharmaceutical syrups through the HPLC-DAD (High Performance Liquid Chromatography with Diode Array Detection) method, and investigates the effects on 5-HMF levels of the 72-hour storage of syrups at temperatures of 40 °C. The 5-HMF level in syrups stored at room temperature varied between 1.34 µg/mL to 15.63 µg/mL, while in syrups stored at higher temperatures, the levels ranged from 2.24 µg/mL to 18.24 µg/mL. This indicated that 5-HMF content in syrups stored at 40 °C was higher than those measured in syrups stored at room temperature, although the increase was not found to be statistically significant ($p>0.05$). In addition to measuring the amount of 5-HMF in pharmaceutical syrups, this study also examined the changes in the levels of this dehydration product in syrup formulations under hot climates and according to storage conditions.

Keywords: 5-hydroxymethylfurfural (5-HMF); pharmaceutical syrup; HPLC-DAD; exposure; sucrose; expiration date.

Submitted: November 02, 2018. **Accepted:** December 28, 2018.

Cite this: Ünüvar S. Determination of 5-hydroxymethylfurfural (5-HMF) in Expired Pharmaceutical Syrups by Using HPLC-DAD Method. JOTCSA. 2018;5(3):1431-40.

DOI: http://dx.doi.org/10.18596/jotcsa.477710.

***Corresponding author. E-mail:** songul.unuvar@inonu.edu.tr. Tel: +90 506 245 40 54, Fax: +90 422 341 12 17.

INTRODUCTION

The compound 5-HMF consists of a furan ring along with aldehyde and alcohol functional groups. Its chemical formula is 5-(hydroxymethyl)-2-furancarboxaldehyde ($C_6H_6O_3$) (1), and it is used in the synthesis of certain organic compounds (2) and novolac resins (3). It is also used as an intermediate substance in the synthesis of some crown ethers (4), and in the production processes of some polymers, surfactants, solvents, pharmaceutics and plant protection agents (5). It is one of the most significant products of non-enzymatic Maillard reaction (6). Upon heating food that contains sugar or carbohydrates, it forms as a result of hexose reduction reaction in the presence of amino-acids or proteins (7).

The presence of 5-HMF has been reported in several foods, including honey, grain products, biscuits, cereals, UHT milk, tomato products, instant coffee, dried fruits, bread, pasta, citrus juices, beer, syrup, jams, canned peach, dried grape, alcohol, apple juice, milk and cereal-based infant formula. The presence of 5-HMF in foods reflects a breakdown or change of substances containing sugar, which is why 5-HMF levels in

food are generally analyzed for quality control purposes (8-12). Food processing conditions, such as temperature, time and water activity, affect 5-HMF content in foods. The daily uptake of 5-HMF in foods may occasionally reach 150 mg/day (13) and it may be present in foods at varying levels (10,14). Very high levels of 5-HMF can be found in such foods as dried fruits or caramelized products (> 1 g/kg) (7). In addition to caramelized foods, 5-HMF has also been identified in caramel-colored pharmaceutical syrups (15). Although the concentrations reported in pharmaceutical syrups are very low, there are concerns about the potential interactions between 5-HMF and functional amino groups of pharmaceutics (11).

The formation of 5-HMF occurs as a result of the acid-catalyzed dehydration process of fructose, sucrose and, to a lesser extent, glucose. As a result, it may, in addition to food, also be found in heat-sterilized parenteral nutritional solutions containing glucose/fructose (16). The quantitative analysis of 5-HMF in clinical research and therapeutics is of great importance as in foods (17). Various methods have been defined for the measurement of 5-HMF levels, including colorimetric, spectroscopic, chromatographic, polarographic and two spectrophotometric methods; White's method and Winkler's method (6,18,19). HPLC method and spectrophotometric methods were recently tested by the International Honey Commission (IHC) (20). The first used before the spectrophotometric methods were optical and chemical methods (17). The basis of the White's method is based on the measurement of UV absorbance of clarified aqueous honey solutions with and without bisulfite. In the other spectrophotometric Winkler method, the UV absorbance of honey solutions with barbituric acid and p-toluidine is measured. Although these two methods are fast, their sensitivity and specificity are not sufficient. In addition, the use of carcinogen p-toluidine in the Winkler's method is a disadvantage. The disadvantage of the HPLC method is that it is more expensive, but it provides advantages in terms of both labor and time (20,21). In the HPLC method is according to Jeuring and Kuppers: firstly honey is dissolved in water. 5-HMF is determined on a reversed phase HPLC column with water and methanol as isocratic mobile phase after millipore filtration (21). Borate is used as supporting electrolyte in electrochemical method. The basis of the method is a single and sharp reduction signal against the silver or silver chloride (22). Yuan *et al.* have used the ion exchange liquid chromatography with photodiode array detection technique (23). Another method used in 5-HMF analysis is the automated flow injection method which provides a detection range of 5-40 ppm (24). Caffeine is used as an internal standard in micellar electrokinetic capillary chromatography, which is used in 5-HMF analysis. This technique allows rapid quantification of the sample, especially in honey without prior pretreatment (25). The real time coupled with time of flight mass spectrometry is another method used in 5-HMF analysis (26). Based on the information from the literature review, we conclude that the differences between the methods cause very low levels of changes in the 5-HMF results. On the other hand, the use of incorrect or inadequate procedures in the 5-HMF determination leads to inaccurate results. We preferred the HPLC-DAD method among these listed methods. Because this method is a rapid, sensitive and automated method that separates 5-HMF from other related compounds in syrup samples and prevent interference in the determination. To our knowledge this is the first study to measure 5-HMF amount in pharmaceutical syrups by using HPLC-DAD method. Apart from syrups, several other pharmaceutical preparations, including tablets, capsules, micropellets, pills, ampules, mouthwashes, pomades and creams, tend to be stored at room temperature, which is defined as 25 °C degrees or lower (15–25 °C). However, in Turkey, ambient temperatures may exceed 40 °C during the summer. It is known that 5-HMF levels increase in foods and pharmaceutical preparations stored at high temperatures and for long durations after production.

The present study investigates the effects of post-expiration temperatures on 5-HMF levels in pharmaceutical syrup formulations exposed to high temperatures after their expiration date.

MATERIALS AND METHODS

Chemicals

The 5-Hydroxymethyl-2-furfural (5-HMF) was obtained from Dr. Ehrenstorfer GmbH (Germany). Methanol was analytical grade and was obtained from Merck (Darmstadt, Germany). Ultrapure water was used in all experiments (Milli-Q system, Millipore, Bedford, MA).

Preparation of stock and standard solutions

On the day of the experiment, 10 mg of high purity (>98%) 5-HMF standard was weighed using an analytic scale and completed to 100 mL with ultrapure water in a 100 mL volumetric flask. This provided 100 ppm of stock standard solution. From this stock standard, 1, 2, 5, 10 and 50 mL solutions were transferred to 100 mL volumetric flasks and the volumes were completed to the mark line using ultrapure water, which provided 1, 2, 5, 10 and 50 ppm standard solutions.

Preparation of samples

The study was carried out using 10 different pharmaceutical syrup samples obtained from the pharmacy. The syrups were kept at room temperature (25±2 °C) for 72 hours. Separate 30 g samples were taken from each syrup and stored for 72 hours in an incubator set to 40±2 °C (Thermo Scientific Heratherm). Then, 10 grams of the samples were taken from the syrups kept at both temperatures and dissolved in 25 mL of

ultrapure water, quantitatively transferred to a 50 mL volumetric flask and completed to 50 mL with ultrapure water. The flasks were placed in a shaker for 15 minutes to ensure complete dissolution of the syrup. Before injection into the HPLC column, the solutions were sampled into syringes and passed through a 0.45 µm filter (SIMPLEPURE) and then transferred to 2 mL amber vials. The 5-HMF samples were kept protected from light and air throughout the study.

Instruments

Chromatography analyses were carried out with an Agilent 1100 HPLC device, which comprises a degasser (G1379A), quaternary pump (G1311A), autosampler (G1313A) and diode array detector (DAD) model G1315. Separations were carried out in an ACE C18 column, 250 x 4.6 mm x 5 µm particle sized. The mobile phase used was methanol: water (90: 10, v/v); the prepared mobile phase was placed into the HPLC device and passed through the column at a flow rate of 1 mL/min to condition the column. The samples transferred to the vials were injected into the HPLC system. Flow rate: 1 mL/min. Injection time: 20 min. The temperature of the column compartment was 25 °C and injection volume was 20 µL. Monitoring of the analytes was carried out using a DAD detector at 284 nm wavelength (27).

The determined limit of detection (LOD, S/N=3) and limit of quantification (LOQ, S/N=10) values for 5-HMF substances were 0.011 µg/mL and 0.036 µg/mL, respectively. The linearity of the method used was tested in the concentration range of 1-50 mg/L by means of an 5-HMF standard (GmbH, Germany).

Statistical analysis

The statistical analysis of the data was carried out using the SPSS Version 11.5 statistical package software (SPSS Inc., Chicago, IL, USA) and was expressed as mean±SD. A Mann-Whitney U-test was used for the comparison of the two independent groups and a Pearson correlation test was used in the evaluation of the correlations. P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The analyzed syrups contained raspberry, orange, grape and mixed-fruit sweeteners, and all of the samples had reached their expiration date (Table 1).

Table 1: Flavor and sweetener contents of the expired pharmaceutical syrups.

Sample name	Expiration date (month)	Sweetener	Flavor
S1	2	Sucrose	-
S2	4	Sucrose	Mixed-fruits
S3	15	Glycerin	Mixed-fruits
S4	11	Glycerin	Orange
S5	12	Sucralose	Mixed-fruits
S6	5	Sucrose	Raspberry
S7	6	Sodium saccharine	Mixed-fruits
S8	4	Sucrose	Orange
S9	15	Sodium saccharine	Raspberry
S10	1	Sucrose	Grape

Figure 1 shows the calibration curve of standard solutions, and the chromatograms of standard solutions of 5-HMF and a syrup sample injected with 5-HMF content. The calibration curve was drawn using peak areas of increasing concentrations of standard solutions (1, 2, 5, 10, and 50 µg/mL) (Figure 1A). Calibration curve of increasing concentrations of 5-HMF standards was drawn by evaluating five replicates of each.

Retention time was estimated as minute 10.038 for the chromatogram of the 10 µg/mL standard solution (Figure 1B). In the syrup sample, the peak level for 5-HMF was attained at minute 10.493 (Figure 1C). 5-HMF was not detected in not expired syrup sample of S7 (Figure 1D).Three replicates of syrup samples were analyzed. The mean 5-HMF level in the syrup samples stored at room temperature was 7.50 µg/mL, while the mean 5-HMF level in the incubated syrup samples was 8.88 µg/mL. A total of four samples were studied from each syrup sample to keep two of them in room temperature and the other two in incubation. Table 2 shows the 5-HMF levels of syrups stored at different temperatures for 72 hours.

Table 2: 5-HMF concentrations in syrups stored at different temperatures (µg/mL).

Sample name	25±2 ºC	40±2 ºC
S1	4.94±0.11	6.54±0.80
S2	6.98±0.14	9.10±0.18
S3	n.d.	n.d.
S4	n.d.	n.d.
S5	1.34±0.31	4.85±1.24
S6	2.87±0.03	2.24±0.20
S7	15.63±0.92	18.24±2.08
S8	10.84±1.37	11.56±1.22
S9	13.21±0.20	13.92±1.33
S10	4.20±0.42	4.59±0.41

n.d: not detected. Results are presented as mean ± SD.

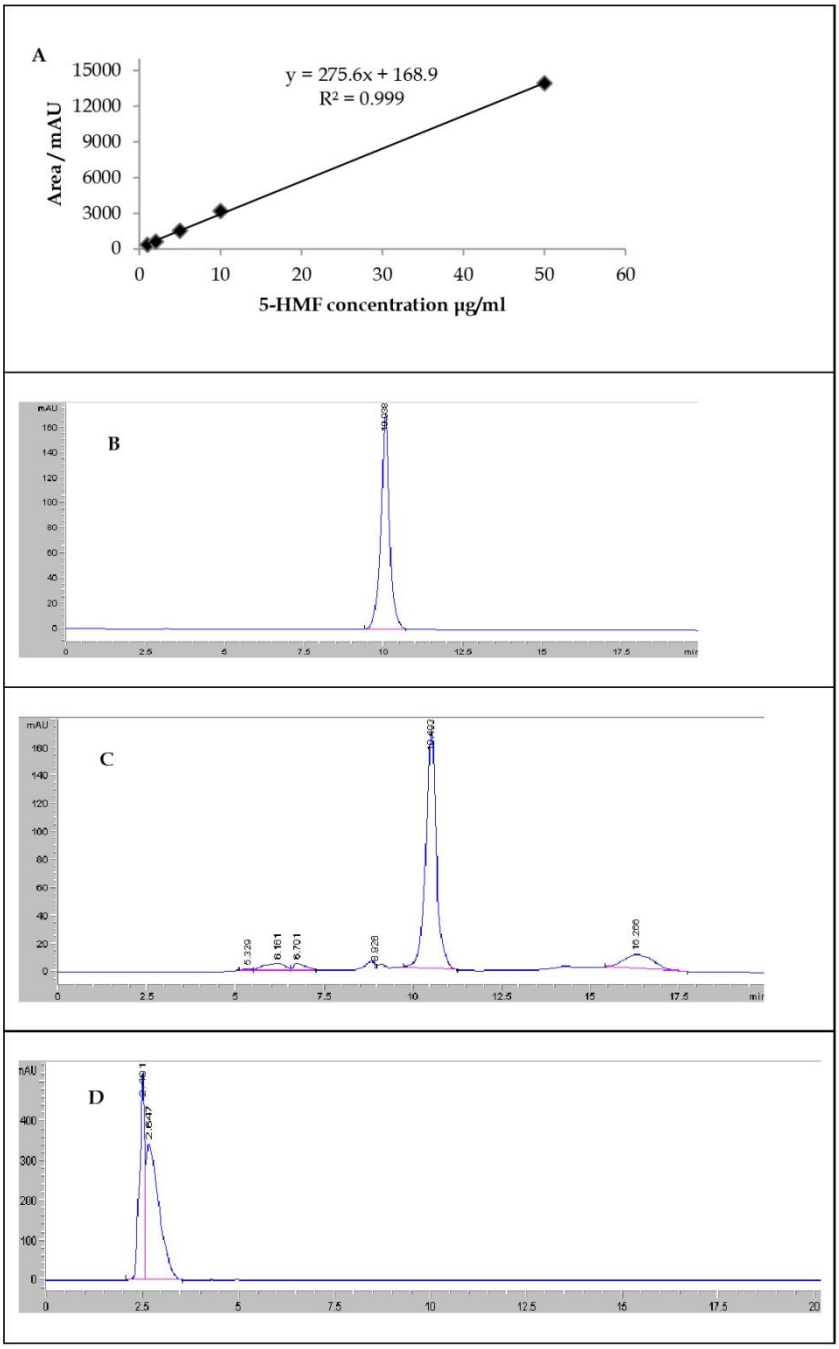

Figure 1. A) Calibration curve of standard solutions of 5-HMF. **B)** Chromatogram of injected standard 5-HMF **C)** Chromatogram of 5-HMF in a expired syrup sample.**D)**HPLC result of not expired pharmaceutical syrup sample.

Although heat treatment increased 5-HMF content in all samples except one, the increases were not statistically significant (p: 0.638).

The relationship between time after expiration and 5-HMF content was evaluated for syrups with different expiration dates (Figure 2). Although a positive correlation was found, the relationship between the variables was weak (r: 0.227) and insignificant (p: 0.588).

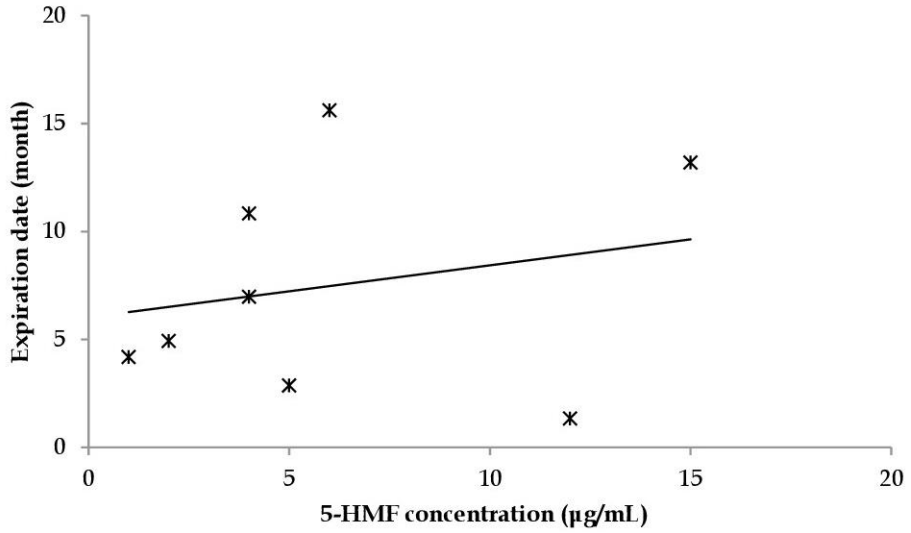

Figure 2. Correlation between time after expiration and 5-HMF content.

Human exposure to 5-HMF essentially occurs through the consumption of processed foods and beverages, the use of pharmaceutical preparations and the inhalation of tobacco smoke. It may also occur occasionally through occupational exposure as a result of the inhalation of the vapor of solutions including 5-HMF, and dermal contact with these compounds in facilities producing or using 5-HMF-derived polymers or chemicals (11).

5-HMF is metabolically activated through the sulfonation of the allylic hydroxyl functional groups by sulfotransferases (SULT1A1), and its bioactivation results in the formation of 5-sulfoxymethylfurfural (SMF) (28,29). It shows no effect in standard genotoxicity tests, but its mutagenic and carcinogenic activities depend on the reactive by product SMF (30). In cell culture studies, it has been reported to have weak genotoxic effects on the HepG2 cell lines (31), and it can also cause DNA damage in cells, irrespective of SULT1A1 activity. However, DNA damage caused by 5-HMF has only been observed in high concentrations. For instance, a significant level of 5-HMF-induced DNA damage was reported after exposure to 5-HMF at a concentration of 100 mM for three hours (28). Both 5-HMF and SMF were found to be weak intestinal carcinogens in mice (32). In addition, high concentrations of 5-HMF have been shown to have irritant effects on the upper respiratory airways, eyes, skin and mucosal membranes. Based on findings obtained from experimental animals, 5-HMF was suggested to have tumorigenic and colon cancer-stimulating effects (6), and some researchers have reported that 5-HMF can act as a neurotoxin, and that its accumulation in the body and interactions with proteins may result in muscle and visceral lesions (33). Taking all this into account, it has become a substance of interest for researchers, and the presence of 5-HMF in foods has raised toxicological concerns (29).

Later studies have suggested that this is not the case, and the carcinogenic activity of 5-HMF has been rejected through direct or indirect investigations (34). Apart from the absence of established genotoxic, mutagenic or carcinogenic activities, some studies in recent years have reported that 5-HMF may even have some favorable effects, for example, as a potential new antioxidant in the fight against cancer (35). It is also shown to have beneficial physiological effects, such as reducing oxidative stress resulting from high glucose levels (36), neuroprotective effects (37), anti-hypoxic effects (38), anti-allergenic effects (39) and anti-inflammatory activity (40).

While several countries have established limits for 5-HMF content in food products, it is important to consider countries with hot climate conditions when defining such limits for 5-HMF content. The 5-HMF limit in honey has been defined as 40 ppm, whereas a level of 80 ppm was considered the standard for countries with hot climates (41). In Turkey, the upper limits for honey, fruit juice and molasses products have been defined as 40 mg/kg, 20 mg/kg, and 75 mg/kg, respectively (42).

While 5-HMF is formed spontaneously, it is generally produced during autoclaving. If pharmaceutical fluids contain glucose, the heat applied during sterilization may cause breakdown and the formation of 5-HMF, and it has been detected in dialysis solutions containing 1 to 60 percent glucose (pH 1-8) that were heat-sterilized at 121 °C (43). The concentrations detected in sterile glucose solutions, intravenous solutions and glucose solutions vary between 1–90 mg/L, 3–56 mg/L and 1–4 mg/L, respectively. 5-HMF concentrations have also been positively correlated with high acidity (pH<4), high sterilization temperatures (>110 °C) and long sterilization times (30 min) (11).

Another study investigated preparations of intravenous injection solutions containing 10 percent fructose, which had a pH lower than 3.5–4.0 and were sterilized at temperatures between 110 and 130 °C. Concentrations of 5-HMF in a newly prepared 50 percent dextrose solution were found to be 0.10 µg/mL, while this level increased to 0.72 µg/mL 24 hours after preparation, and following four years of storage at 70 °F (21.1 °C), 5-HMF levels increased to 5.8 µg/mL (44).

Fruits contain varying amounts of 5-HMF due to different sugar and organic acid contents (citric, malic, and ascorbic acids, etc.). Both the highest and the lowest 5-HMF levels were found in syrup samples with mixed fruit as flavor agent. Also the second lowest and the second highest levels were found in grape flavored syrups. On the other hand, 5-HMF was not detected in syrup samples containing glycerin as a sweetener. 5-HMF is produced as a result of dehydration of fructose, glucose, and other reducing sugars and in the early stages of the Maillard reaction. Therefore, 5-HMF formation is not expected in the syrup samples containing glycerin. The reason for using two syrup samples containing glycerin in this study is to contribute to this situation. The results show that there is no effect of flavors on 5-HMF levels.
A positive correlation has been noted in the evaluation of 5-HMF level and expiration date, although this cannot be directly associated with the expiration date, as syrup samples contain different types and amounts of sweeteners. There is also the same situation in food products not only in pharmaceutical formulations. The formation of 5-HMF increases in parallel with the storage time of foods.

High temperatures induce the formation of 5-HMF. Aside for in one sample, the 5-HMF content in syrups kept at 40±2 °C was found to be higher than those kept at room temperature. But this increase was not statistically significant ($p>0.05$). There may be a significant increase in the longer incubation time at higher temperatures. However, our aim was to show that the level could be increased even in short-term small temperature increases.

The highest levels were found in two samples that sodium saccharin used as a sweetener. 5-HMF commonly occurs by the dehydration of monosaccharides and disaccharides. A study investigated the effects of different temperatures and pH values on 5-HMF formation while preparing simple syrups, and based on the findings, the authors concluded that pH and storage temperature had significant effects on 5-HMF formation. Accordingly, the best way to avoid 5-HMF formation is to prepare the syrup under high pH conditions while maintaining low storage temperatures, although there have been no studies to date concerning the effects of pH and temperature on 5-HMF formation in syrups (16).

The presence of 5-HMF and similar compounds in some parenteral preparations containing dextrose, solutions prepared for peritoneal dialysis and also some excipients used in pharmaceutical sector is a marker of quality for pharmaceutical products. Based on European Pharmacopeia, the level of dextrose in solutions used for peritoneal dialysis should not exceed 10 µg 5-HMF and 25 mg dextrose if they contain no bicarbonate, and 20 µg 5-HMF and 25 mg dextrose if they contain bicarbonate (45). While 5-HMF limits are set by the relevant regulations, factors such as technological processes, the sugar content of medical products, and the storage conditions of foods and pharmaceutical products should be considered.

CONCLUSION

The storage conditions of medications are generally defined according to mild climatic conditions and low sunlight. If extremely hot climatic conditions are not taken into account, the stability of medications may become a source of significant problems in countries with high temperature and sunlight levels. None of the syrup samples we analyzed were contain 5-HMF above the legal limits set for foods. Although 5-HMF content was not found to be high in the syrups analyzed in the present study, there may still be interactions with the amino groups in pharmaceutical formulations, and changes might be seen in the activity of such drug products. Accordingly, even insignificant amounts of 5-HMF and similar by products in medical formulations and foods should not be ignored. 5-HMF levels can exceed legal limit in several food items. The only food for which a legal limit on 5-HMF concentrations has been set is honey. 5-HMF may exceed tolerable daily intake as a result of taking from different sources such as foods and medicine. Therefore monitoring of 5-HMF contents in foods and medical products used by sensitive populations, especially children seems to be necessary. Syrups containing sweeteners were used in our study. This type of syrups are mostly used by children. Other 5-HMF sources include sugar-containing foods such as honey, jam, fruit juices, which children consume frequently. A low concentration of 5-HMF does not mean that it does not cause toxicity. Because it can accumulate during both nutrition and medical treatment.

REFERENCES

1. National Center for Biotechnology Information. PubChem Compound Database; CID=237332, https://pubchem.ncbi.nlm.nih.gov/compound/237332 (accessed July 3, 2018).

2. Zakrzewska ME, Bogel-Lukasik E. Bogel-Lukasik R. Ionic Liquid-Mediated Formation of 5-hydroxymethylfurfural - a Promising Biomass-

Derived Building Block. Chem Rev. 2011;111(2):397-417.

3. Brode GL, Mark HF, Othmer DF, Overberger CG, Seaborg GT, Grayson M. In Encyclopedia of Chemical Technology, Kirk RE, Othmer DF, Eds. Wiley, New York, 1982;17:411-3.

4. Larousse C, Rigal L, Gaset A. Synthesis of 5,5'-oxydimethyl bis (2-furfural) by Thermal Dehydratation of 5-hydroxymethyl Furfural in the Presence of Dimethylsulfoxide. J Chem Technol Biotechnol. 1992;53(1):111-6.

5. Kunz M. Hydroxymethylfurfural, a Possible Basic Chemical Industrial Intermediates. In Inulin and Inulin-Containing Crops, Fuchs A, Ed. Elsevier Science Publishers, Amsterdam, The Netherlands, 1993;3:149-60.

6. Morales FJ. Hydroxymethylfurfural (HMF) and related compounds. Process-Induced Food Toxicants: Occurence, Formation, Mitigation and Health Risks, (eds: Stadler, R H, Lineback DR), A john Wiley & Sons Inc Publications, 2009.

7. Ruiz-Matute AI, Weiss M, Sammataro D, Finely J, Sanz ML. Carbohydrate Composition of High-Fructose Corn Syrups (HFCS) Used for Bee Feeding: Effect on Honey Composition. J Agric Food Chem. 2010;58(12):7317-22.

8. Arribas-Lorenzo G, Morales FJ. Estimation of Dietary Intake of 5-hydroxymethylfurfural and Related Substances From Coffee to Spanish Population.Food Chem Toxicol. 2010;48(2):644-9.

9. Husøy T, Haugen M, Murkovic M, Jöbstl D, Stølen LH, Bjellaas T, Rønningborg C, Glatt H, Alexander J. Dietary Exposure to 5-hydroxymethylfurfural From Norwegian Food and Correlations with Urine Metabolites of Short-term Exposure. Food Chem Toxicol. 2008;46(12):3697-3702.

10. Rufían-Henares JA, de la Cueva SP. Assessment of Hydroxymethylfurfural Intake in the Spanish Diet. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008;25(11):1306-12.

11. NTP, Testing Status of Agents at NTP. 5-(Hydroxymethyl)-2-furfural. Nomination Background. Washington, DC: National Toxicology Program, Dept Health Human Serv. https://ntp.niehs.nih.gov/testing/noms/search/summary/nm-n95002.html (accessed May 18, 2018).

12. Gökmen V, Senyuva HZ. Improved Method for the Determination of Hydroxymethylfurfural in Baby Foods Using Liquid Chromatography-mass Spectrometry. J Agric Food Chem. 2006;54(8):2845-9.

13. Delgado-Andrade C, Seiquer I, Navarro MP, Morales FJ. Maillard Reaction Indicators in Diets Usually Consumed by Adolescent Population. Mol Nutr Food Res. 2007;51(3):341-51.

14. Abraham K, Gürtler R, Berg K, Heinemeyer G, Lampen A, Appel KE. Toxicology and Risk Assessment of 5-Hydroxymethylfurfural in Food. Mol Nutr Food Res. 2011;55(5):67-78.

15. Hewala II, Blaih SM,nZoweil AM, Onsi SM. Detection and Determination of Interfering 5-hydroxymethylfurfural in the Analysis of Caramel-coloured Pharmaceutical Syrups. J Clin Pharm Ther. 1993;18(1):49-53.

16. Ghaderi F, Shadbad MRS, Hoseinzadehm. Effect of pH and Storage Temperature on 5-(hydroxymethyl) Furfural (5HMF) Formation in USP Syrup Preparation. Pharm Sci. 2015;21(1):1-5.

17. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) Levels in Honey and Other Food Products: Effects on Bees and Human Health. Chem Cent J. 2018;12(1):35.

18. Zappalà M, Fallico B, Arena E, Verzera A. Methods for the Determination of HMF in Honey: a Comparison. Food Control. 2005;16(3):273-7.

19. Sivakesava S, Irudayaraj J. A Rapid Spectroscopic Technique for Determining Honey Adulteration with Corn Syrup Direct Electrochemical Determination of Hydroxymethylfurfural (HMF) and its Application to Honey Samples. J Food Sci. 2001;66(6):787-92.

20. IHC, The International Honey Commission. Bogdanov S. 1999; 1–54. http://www.ihc-platform.net/ihcmethods2009.pdf (accessed October 18, 2018).

21. Wootton M, Ryall L. A Comparison of Codex Alimentarius Commission and HPLC Methods for 5-hydroxymethyl-2-furaldehyde Determination in Honey. J Apic Res. 1985;24(2):120124.

22. Reyes-Salas EO, Manzanilla-Cano JA, Barceló-Quintal MH, Juárez-Mendoza D, Reyes-Salas M. Direct Electrochemical Determination of Hydroxymethylfurfural (HMF) and its Application to Honey Samples. Anal Lett. 2006;39(1):161-171.

23. Yuan JP, Chen F. Separation and Identification of Furanic Compounds in Fruit Juices and Drinks by High-performance Liquid Chromatography Photodiode Array Detection. J Agric Food Chem. 1998;46:1286-91.

24. De la Iglesia F, Lázaro F, Puchades R, Maquieira A. Automatic Determination of 5-hydroxymethylfurfural (5-HMF) by a Flow Injection Method. Food Chem. 1997;60(2):245-50.

25. Rizelio VM, Gonzaga LV, Borges GdSC, Micke GA, Fett R, Costa ACO. Development of a Fast MECK Method for Determination of 5-HMF in Honey Samples. Food Chem. 2012;133:1640-5.

26. Rajchl A, Drgová L, Grégrová A, Čížková H, Ševčík R, Voldřich M. Rapid Determination of 5-hydroxymethylfurfural by DART Ionization with Time-of-flight Mass Spectrometry. Anal Bioanal Chem. 2013;405:4737-45.

27. Jeurings J, Kuppers F. High Performance Liquid Chromatography of Furfural and Hydroxymethylfurfural in Spirits and Honey. J Assoc Off Anal Chem. 1980;63(6):1215-8.

28. Durling LJ, Busk L, Hellman BE. Evaluation of the DNA Damaging Effect of the Heat-induced Food Toxicant 5-hydroxymethylfurfural (HMF) in Various Cell Lines with Different Activities of Sulfotransferases. Food Chem Toxicol. 2009;47(4):880-4.

29. Murkovic M, Pichler N. Analysis of 5-hydroxymethylfurfual in Coffee, Dried Fruits and Urine. Mol Nutr Food Res. 2006;50(9):842-6.

30. Bakhiya N, Monien B, Frank H, Seidel A, Glatt H. Renal Organic Anion Transporters OAT1 and OAT3 Mediate the Cellular Accumulation of 5 Sulfooxymethylfurfural, a Reactive, Nephrotoxic Metabolite of the Maillard Product 5-hydroxymethylfurfural. Biochem Pharmacol. 2009;78(4):414-9.

31. Severin I, Dumont C, Jondeau-Cabaton A, Graillot V, Chagnon MC. Genotoxic Activities of the Food Contaminant 5-hydroxymethylfurfural Using Different in Vitro Bioassays. Toxicol Lett. 2010;192(2):189-94.

32. Svendsen C, Husoy T, Glatt H, Paulsen JE, Alexander J. 5-Hydroxymethylfurfural and 5-Sulfooxymethylfurfural Increase Adenoma and Flat ACF Number in the Intestine of Min/+ Mice. Anticancer Res. 2009;29(6):1921-6.

33. Li Y, Lu X. Investigation on the Origin of 5-HMF in Shengmaiyin Decoction by RP-HPLC Method. J Zhejiang Univ Sci B. 2005;6(10):1015-21.

34. Michail K, Matzi V, Maier A, Herwig R, Greilberger J, Juan H, Kunert O, Wintersteiger R. Hydroxymethylfurfural: an Enemy or a Friendly Xenobiotic? A Bioanalytical Approach. Anal Bioanal Chem. 2007;387(8):2801-14.

35. Zhao L, Chen J, Su J, Li L, Hu S, Li B, Zhang X, Xu Z, Chen T. In vitro Antioxidant and Antiproliferative Activities of 5-hydroxymethylfurfural. J Agric Food Chem. 2013;61(44):10604-11.

36. Cao G, Cai H, Cai B, Tu S. Effect of 5-hydroxymethylfurfural Derived from Processed Cornus Officinalis on the Prevention of High Glucose-induced Oxidative Stres in Human Umbilical Vein Endothelial Cells and Its Mechanism. Food Chem. 2013;140(1-2):273-9.

37. Liu A, Zhao X, Li H, Liu Z, Liu B, Mao X Guo L, Bi K, Jia Y. 5-Hydroxymethylfurfural, an Antioxidant Agent from Alpinia Oxyphylla Miq. İmproves Cognitive Impairment in Aβ 1–42 Mouse Model of Alzheimer's Disease. Int Immunopharmacol. 2014;23(2):719-25.

38. Li MM, Wu LY, Zhao T, Xiong L, Huang X, Liu ZH Fan XL, Xiao CR, Gao Y, Ma YB, Chen JJ, Zhu LL, Fan M. The Protective Role of 5-HMF Against Hypoxic Injury. Cell Stress Chaperones 2011;16(3):267-73.

39. Yamada P, Nemoto M, Shigemori H, Yokota S, Isoda H. Isolationof 5-(hydroxymethyl) Furfural from Lycium Chinense and its Inhibitory Effect on the Chemical Mediator Release by Basophilic Cells. Planta Med. 2011;77(5):434-40.

40. Alizadeh M, Khodaei H, Mesgari Abbasi M, Saleh-Ghadimi S. Assessing the Effect of 5-hydroxymethylfurfural on Selected Components of Immune Responses in Mice Immunised with Ovalbumin. J Sci Food Agric. 2017;97(12):3979-84.

41. JECFA, Joint of FAO/WHO Food Standards Ppogramme Codex Committee on Sugars Seventh Session London, United Kingdom, 9-11 February 2000. www.fao.org/input/download/standards/310/cxs_012e.pdf (accessed January 11, 2018).

42. TFC. Turkish Food Codex, Meyve Suyu ve Benzeri Ürünler Tebliği, Tebliğ No: 2014/34. http://www.resmigazete.gov.tr/eskiler/2014/08/20140806-17.htm. (accessed January 18, 2018).

43. Kjellstrand P, Martinson E, Wieslander A, Kjellstrand K, Jeppsson E, Svensson E, Järkelid L, Linden T, Olsson LF. Degradation in Peritoneal Dialysis Fluids may be Avoided by Using Low pH and High Glucose Concentration. Perit Dial Int. 2001;21(4):338-44.

44. Ulbricht RJ, Northup SJ, Thomas JA. A Review of 5-hydroxymethylfurfural (HMF) in Parenteral Solutions. Fundam Appl Toxicol. 1984;4(5):843-53.

45. European Council Directive 2001/110/EC of 20.12.2001. European Pharmacopoeia 8.0,

Council of Europe. European Directorate for the Quality of Medicines and Health Care. https://www.fsai.ie/uploadedFiles/Consol_Dir2001_110.pdf. (accessed April 7, 2018).