MARMARA
PHARMACEUTICAL JOURNAL

Research Article

www.marmarapharmj.com

Determination and Safety Evaluation of Furfural and Hydroxymethylfurfural in Some Honey Samples by Using a Validated HPLC-DAD Method

Ebru TÜRKÖZ ACAR [1], Sinem HELVACIOĞLU [2], Mohammad CHAREHSAZ [2], Ahmet AYDIN [2] *

[1] Department of Analytical Chemistry, Faculty of Pharmacy, Yeditepe University, Ataşehir 34755 İstanbul, Turkey.
[2] Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Yeditepe University, Ataşehir 34755 İstanbul, Turkey.
* Corresponding Author. E-mail: ahmet.aydin@yeditepe.edu.tr; Tel. +90-216-578 00 00; ORCID No: 0000-0003-3499-6435.

Received: 14 January 2018 / Revised: 31 May 2018 / Accepted: 31 May 2018

ABSTRACT: A novel high performance liquid chromatography method has been developed and validated for the simultaneous determination of furfural and hydroxymethylfurfural in 18 honey samples. An Agilent Poroshell 120 EC-C18 150x3 mm 2.7µm particle sized column and isocratic elution with a 0.5 mL/min flow rate were used. The mobile phase was 10mM pH 2.5 phosphate buffer and acetonitrile and monitoring of analytes was carried on using a DAD detector at 284 nm wavelength. The method was validated according to USP guideline in terms of accuracy, precision, specificity, linearity and range\ robustness and ruggedness. According to the obtained results, the concentration levels of hydroxymethylfurfural were between 19.56-209.42 mg/kg in honey samples. Observed concentration values of HMF for 5 honey samples were higher than requirements and the highest level of hydroxymethylfurfural was observed in a thyme honey sample (209.42 mg/kg). The concentration values of furfural found in honey samples were in the range of 0.34-2.23 mg/kg. The highest level of furfura was determined in the thyme honey sample (2.23 mg/kg) also containing the highest concentration of hydroxymethylfurfural. In this study, the margins of exposure to furfural were also calculated for investigated honey samples. The margins of exposure for all analyzed samples were above the value of 100, indicating the safety of samples regarding to furfural exposure. The excessive hydroxymethylfurfural contents in some samples is a concerning point for public health and the national authority needs to increase its supervision on the honey.

KEYWORDS: High performance liquid chromatography; honey; furfural; hydroxymethylfurfural.

1. INTRODUCTION

The Maillard reaction is a chemical reaction between amino acids and reducing sugars and occurs in food storage at low temperature as well as during cooking conditions [1]. The reaction takes place in all the foods that are baked (bread, cookies, cakes, etc.), fried (meat, potato chips etc.) and heat-treated during and/or after production throughout the shelf life (honey, molasses, coffee, jam etc.) [2]. Furan derivatives, furfural (2-furaldehyde, F) and hydroxymethylfurfural (5-hydroxy-2-furaldehyde, HMF) are important Maillard reaction products which are present in numerous foodstuffs at high levels.

HMF is an indicator of quality in several food products and there is an HMF content limitation for some foods such as molasses and honey because of its adverse effects on human health like cytotoxic, mutagenic, genotoxic and carcinogenic consequences [3]. It was shown that HMF at high concentration is cytotoxic and irritant to eyes, skin and mucous membranes [4]. Some studies revealed that HMF may act as both an initiator and a promoter in colon cancer in rats [5, 6]. HMF was investigated for carcinogenicity within the National Toxicology Program (NTP) of the USA and it was found to be carcinogenic based on increased incidences of hepatocellular adenoma [7]. Although, HMF was negative in the classic genotoxicity test battery conducted by various groups, it was mutagenic and genotoxic in genetically modified Salmonella strains [8] and V79 cell lines [9].

F was also investigated for carcinogenicity by oral administration in vivo. It increased the incidence of hepatocellular adenomas and carcinomas in mice, while in rats had a low incidence of cholangiocarcinoma,

How to cite this article: Türköz Acar E, Helvacıoğlu S, Charehsaz M, Aydın A. Determination and Safety Evaluation of Furfural and Hydroxymethylfurfural in Some Honey Samples by Using a Validated HPLC-DAD Method. Marmara Pharm J. 2018; 22 (4): 519-527.

https://doi.org/10.12991/jrp.2018.93
ISSN: 1309-0801

which occur rarely. In another study, gene mutation, sister chromatid exchange and chromosomal aberrations were induced by F in mammalian cells in vitro [10].

These ongoing reports about toxicity of furan derivatives, make the determination of these compounds crucial in different matrices such as foods and beverages.

Human beings consume honey as a sweetener and food source. There have been numerous studies on the nutritional and medicinal properties of honey. However, honey may contain compounds that may lead to toxicity. A heat treatment is applied to honey to facilitate the filtration process, reduce viscosity, delay the crystallization and prevent fermentation during honey processing. Due to this application, furan derivatives may be composed in honey [11]. Also usage of evaluation of HMF level is well known procedure to investigate the quality of honey. However, F is widely accepted as an indicator of flavor changes [11,12].

When concerns of the scientific community about the potential toxicity of furan derivatives are taken into account, new methods were developed to monitor these compounds in different foodstuffs. Developed analytical methods used different techniques such as high performance liquid chromatography (HPLC) [13,14], gas chromatography [15], gas chromatography–mass spectrometry [16,17], high-performance thin-layer chromatography (HPTLC) [18] and micellar elektrokinetic capillary chromatography [19].

However, there are a few studies devoted to analytical validation of these methodologies, especially for honey. Truzzi et al [14] determined the HMF concentration in honey samples and investigating of analytical method is not meet the any validation guide criteria. Bahaffi et al [17] measured HMF levels in some honey samples by using GC-MS but they did not investigate any validation parameters for the method. In another study, HMF was monitored in honey samples by HPTLC, which is a planar chromatography technique, and there was not any validation experiments meeting all criteria [18]. Zappala et al [20] made a comparison between an HPLC method and a spectrophotometry method but they did not indicate any result for validation of analytical methods. In addition, these methods reported LOD and LOQ values between 0.09-6 mg/kg and 0.27-18 mg/kg, respectively. These values can not indicate the HMF concentration values for foods containing little amount of HMF. This situation affects the people consuming continuously this type of foods. As known well, investigating procedure of honey quality needs some additional criteria different from HMF concentration level. Thus, ability of determination of other furan derivatives simultaneously with HMF is very important and can present a wide perspective to analyst. However, the methods in the literature investigated only HMF analysis in the honey samples. In addition, the use of different analytical methods for HMF determination and the use of inaccurate or inadequate procedures are actually a problem. In this study, it was aimed to develop and validate a sensitive, precise and accurate method for the simultaneous determination of HMF and F levels in different honey samples using an HPLC-DAD method. The results also evaluated from toxicological viewpoint in this study.

2. RESULTS AND DISCUSSION

2.1. Optimization of the method

In this study, a new HPLC-DAD method was developed to analyze HMF and F being derivatives of furan. When the sources from the literature were investigated, it was seen that these substances were separated by using a C18 column and water and acetonitrile mixture as mobile phase [21-23] . For this reason, the first studies were made on classical C18 columns having properties 50-250 mm length and 3- 4.6 mm thickness and 2.7-5 µm particle size with water-acetonitrile mixture. However, results obtained from C18 columns were not satisfactory. Thus, a new generation shell column, Agilent Poroshell 120 EC-C18 3.0x 150 mm 2.7µm column was tried for separation. Obtained chromatograms showed that the separation was perfect in view of peak separation, peak shapes and signal values. Further experimental parameters such as flow rate, temperature and mobile phase rate were investigated using this column and water acetonitrile mixture as mobile phase. According to the literature, preparation of samples having a rich matrix need adding some chemical agents such as Carrez solutions (Carrez I-$K_4Fe(CN)_6$ and Carrez II -$Zn(CH_3COO)_2$) to clean sample from the interferences [21, 24, 25]. After adding of Carrez solution, a precipitate was composed and supernatant was used for analysis.

However, after injection of the samples, the pressure of the column was increased to up limit and cleaning procedures were not enough to open the column plug. At this point, it was thought that cations coming from the Carrez solutions could be precipitate as hydroxides at normal water pH conditions. Column working limit was in the range between pH 2-8. By viewing column condition, 50 mM phosphate buffer (pH 2.5) was tried as mobile phase to prevent precipitation of these cations. After this experiment, it was seen that the column pressure decreased in a short time. Due to this situation, 50 mM phosphate buffer pH 2.5 solution

was used for mobile phase A. Different rates of ACN solution were investigated to obtain the optimum separation conditions. ACN rate was decided as 5 percent against 50 mM phosphate buffer pH 2.5 solution due to separated HMF and F peaks as well-shaped and symmetrical by using this system. The obtained chromatogram of the F and HMF standard mixture is presented in Figure 1.

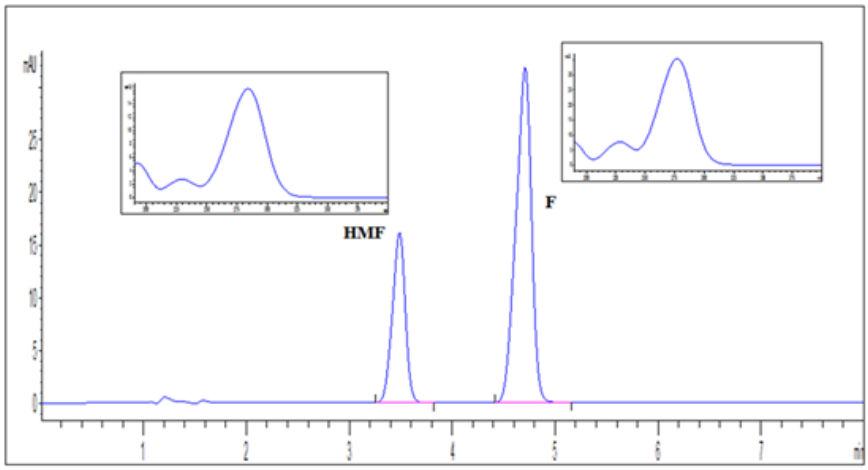

Figure 1. Chromatogram of the standard mixture of HMF and F at a concentration value of 0.5 ppm. F: furfural; HMF: hydroxymethylfurfural. Small shapes are UV spectrums which were obtained from chromatograms by using Chem Station software at peak maximum points.

After the optimization procedure, the experiments related with validation of the method was started. Dilution of the solutions was made with 50 mM pH 2.5-phosphate buffer solution to obtain unity of the honey samples and standard solutions.

2.1.1. System suitability

System suitability test was performed for the developed method. Obtained results are presented in Table 1 and it can be said that the method developed is appropriate to analyze the F and HMF derivatives.

Table 1. System suitability test results for 0.8 ppm standard mixture of F and HMF (n=6).

Parameter	HMF	F
Retention time (min)	4.027	5.345
Peak Area (mAu x min)	1073.8	900.5
RSD value of peak area (≤1%)	0.11	0.22
k', Capacity factor (≥2)	2.02	2.67
USP Tailing (≤2)	1.24	1.27
N, Number of theoretical plate (≥2000)	5018	6412
Resolution (≥2)	5.34	5.34

F: furfural.
HMF: hydroxymethylfurfural.

2.1.2. Calibration curve

Different concentration values of each compound were investigated to determine dynamic range for the developed method. For this purpose, standard solutions of each analyte as a mixture were prepared daily by diluting from stock solution of compounds. Chromatograms obtained for each standard mixture were recorded and investigated to determine calibration parameters of the method. Calibration curve dynamic ranges and related method limits are shown in Table 2.

Table 2. Calibration curve parameters of the method developed for each analyte.

Parameter	HMF	F
LOD (ppm)	0.007	0.005
LOQ (ppm)	0.02	0.01
Dynamic Range (ppm)	0.1-10	0.1-10
Slope	279.35	724.00
Intercept	-1.699	-26.146
R^2	0.9999	0.9999

LOD: Limit of detection.
LOQ: Limit of quantification.
F: furfural.
HMF: hydroxymethylfurfural.

2.1.3. Accuracy

Recovery and relative standard deviation values are presented in Table 3. Accordingly, it is seen that recovery values are in 95-105 % range. This situation shows that the method provides needed accuracy of the measurements.

2.1.4. Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation (RSD) for a statistically significant number of samples. Table 3 also shows precision of the method due to presentation of RSD values obtained from three repetitive analysis of known amount of standards at three different level. RSD values of intra-day studies are lower comparing inter-day studies. Obtained results shows that the developed method provides the precision of the measurements.

Table 3. Results of accuracy and precision study for the developed method.

Analyte	Concentration Level	Intraday (n=3)		Inter day (n=9)	
		Recovery%	RSD%	Recovery%	RSD%
HMF	**L**	96.12	0.27	97.89	1.51
	M	101.63	0.53	100.89	0.53
	H	100.93	0.08	100.53	0.45
F	**L**	103.45	0.14	101.88	1.46
	M	100.07	0.12	101.27	0.58
	H	103.23	0.11	100.71	1.34

L: Low-level QC (0.8 ppm).
M: Medium level QC (3 ppm).
H: Hıgh level QC (8 ppm).
F: furfural; HMF: hydroxymethylfurfural.

2.1.5. Specifity

It was observed that materials being in honey samples do not present overlapping peaks with HMF and F. In addition, observed peaks were investigated by comparing online UV spectrums obtained from chromatograms of standard solution and chromatograms of honey samples.

2.1.6. Robustness and ruggedness

The developed method was evaluated in view of robustness and ruggedness. Some experimental parameters were changed deliberately in accordance with this purpose and this change was limited ± 10% of optimized experimental value. Effect of change of experimental values on accuracy and precision was investigated. In this part of the study, temperature, buffer to acetonitrile ratio and pH parameters were changed between limit values. Obtained results are presented in Table 4.

Table 4. Experimental results of robustness and ruggedness studies (n=3).

		Temperature (°C)		Buffer:ACN Rate		pH of Buffer	
Analyte	**Conc. Level**	**27**	**23**	**96:4**	**94:6**	**2.3**	**2.7**
HMF	**L**	100.99±0,58	101.84±0.12	101.35±0.34	104.01±0.76	101.37±0.63	99.16±0.56
	M	100.80±0.18	100.45±0.02	100.97±0.30	100.67±0.08	100.92±0.05	99.45±0.43
	H	101.07±0.18	101.32±0.15	101.08±0.20	101.09±0.09	101.52±0.04	101.23±0.09
F	**L**	106.42±0.10	106.58±0.19	106.50±0.02	110.94±0.41	100.65±0.18	111.91±0.17
	M	104.12±0.29	104.51±0.36	104.57±0.11	104.88±0.03	100.85±0.20	105.53±0.23
	H	100.07±0.01	99.91±0.21	100.01±0.06	99.88±0.02	97.37±0.03	100.01±0.08

L: Low-level QC (0.8 ppm).
M: Medium level QC (3 ppm).
H: Hıgh level QC (8 ppm).
F: furfural; HMF: hydroxymethylfurfural.

In general, when the obtained recovery values were investigated, it can be seen that recovery values were appropriate to 85-115 % percentage rule and results were repeatable. These deliberately changes on experimental parameters indicated that analyst should be more careful on F analysis. Because, calculated recovery values of F versus changed parameters show differences by comparing recovery values of HMF. On the other hand, pH was the most effective parameter on recovery values. Due to this reason, analyst working with the developed method should be sensitive on preparing of buffer and adjusting buffer pH. However, quality control samples should be used for each analysis to eliminate any error.

2.2. Analysis of HMF and F in honey samples

In order to verify the pertinence of the optimized and validated HPLC method in the food quality control, eighteen honey samples of different origins and manufacturers were analyzed in this study. Data are presented as means of triplicates and standard deviations (Table 5) and the example of the sample chromatogram is given in Figure 2. Determination of the peak on obtained chromatogram was made by comparing UV spectrums of peaks. For this purpose, standard solution of F and HMF was injected to the device. A chromatogram of two analytes was obtained as shown in Figure 1. ChemStation software of chromatography device allowed to us investigating of UV spectrums at any time point. UV spectrums of F and HMF were loaded from the software and recorded. After analysis of the honey samples, loaded UV spectrums of expected peaks on chromatogram were loaded and compared with previous spectrums as shown in Figure 2. If the amount of the analyte was appropriate, a good spectrum similar to standard material spectrum was obtained. If the amount of the analyte was at low concentration level, there was little effects on spectrum due to matrix. Hovewer, the spectrums allowed to quantify the related analytes.

Table 5. Analysis results of some marketed and local honey samples (mg/kg).

Sample No	Level of HMF	Level of F	Sample No	Level of HMF	Level of F
1	27.749 ± 0.012	0.365 ± 0.008	**10**	16.367 ± 0.012	0.604 ± 0.003
2	55.412 ± 0.177	1.019 ± 0.007	**11**	12.578 ± 0.010	0.555 ± 0.001
3	25.031 ± 0.021	0.419 ± 0.001	**12**	20.863 ± 0.025	0.399 ± 0.009
4	64.124 ± 0.013	0.423 ± 0.007	**13**	19.564 ± 0.010	0.752 ± 0.002
5	37.631 ± 0.223	0.351 ± 0.011	**14**	24.649 ± 0.055	0.360 ± 0.001
6	45.209 ± 0.030	0.470 ± 0.005	**15**	22.673 ± 0.019	0.344 ± 0.001
7	209.416 ± 0.016	2.226 ± 0.002	**16**	68.825 ± 0.059	0.515 ± 0.001
8	27.692 ± 0.005	0.742 ± 0.002	**17**	20.442 ± 0.025	0.345 ± 0.001
9	24.571 ± 0.013	0.469 ± 0.001	**18**	32.987 ± 0.039	0.401 ± 0.001

F: furfural; HMF: hydroxymethylfurfural.
Results were expressed as the mean ± standard deviation (S.D).

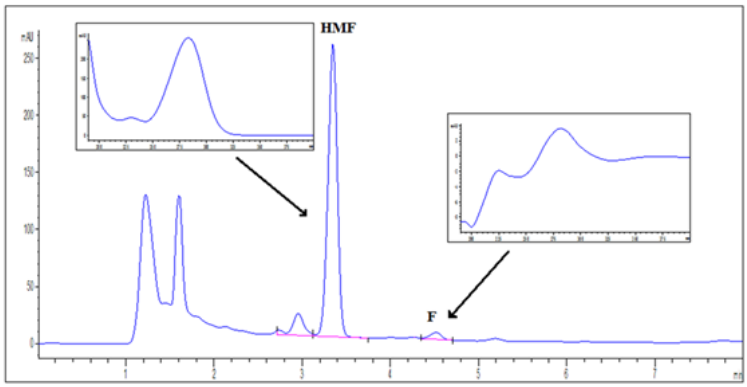

Figure 2. Chromatogram of Sample at optimized conditions: 50 mM pH 2.5 phosphate buffer: ACN (95:5) at s 0.5 mL/min flow rate on Agilent Poroshell 120 EC-C18 150x3 mm 2.7 µm particle sized column and Agilent Poroshell 120 EC-C18 3x5mm guard column, temperature of the column compartment was 25°C and injection volume was 20 µL. DAD detector was adjusted to 284 nm wavelength. F: furfural; HMF: hydroxymethylfurfural.

The levels of HMF were distributed over a wide range of concentrations (19.56-209.42 mg/kg) in honey samples. Highest level of HMF was observed in a thyme honey sample (209.42 mg/kg). Besides this, for five samples the levels observed for HMF were higher than requirements (40 mg/kg) established by Turkish and international standards for honey [26, 27].

There is no F limit established for honey that implies that it should be absent. However, the levels of F in honey samples investigated in this study were in the range of 0.34-2.23 mg/kg. Highest level of F was determined in the thyme honey sample (2.23 mg/kg) which was also contained highest concentration of HMF (209.42 mg/kg).

For the natural occurrence of F in food, Flavor and Essence Manufacturers Association (FEMA) calculated a total potential daily intake of approximately 0.3 mg/kg bw/day for F and precursors of F from natural occurrence in food. Consumption of 10 g honey by a 60 kg person results in intake of 0.00006 to 0.0004 mg/kg bw/day for F, which is far below the potential daily intake limit (0.3 mg/kg bw/day).

The repeated dose toxicity data on F are sufficient for the repeated dose toxicity endpoint. In a study, rats were given microencapsulated F in the diet for 90 days. The NOAEL in that study was established as 53 mg/kg bw/d [28].

In present study, Margin of Exposure (MOE) was calculated to determine human health risk from exposure to the furfural from investigated honey samples. The MOE is a ratio of the toxicity effect level (NOAEL) to the estimated exposure dose and low risk is implied when MOE > 100. Considering the consumption of 10 g honey by a 50 kg person, the MOE for F in investigated honey samples was at the range of 119-755, which provided a MOE > 100 [29]. However, it should be noted that the food is not the only source of exposure to F especially for workers in occupational settings such as pesticide manufacturers, fuel, pulp and paper industries. Therefore, for the risk evaluation the combined exposure to F should be taken into the consideration.

4. CONCLUSION

This study describes a HPLC-DAD method for the determination of HMF and F in foods like honey. The described method is accurate and reliable, and should be applicable to monitoring of HMF and F levels in food. The HMF content of 6 samples from 18 analyzed honey samples were above the requirements established by Turkish and international standards for honey. This excessive HMF contents is a concerning point for public health and the national authority needs to increase its supervision on the production of honey.

5. MATERIALS AND METHODS

5.1. Chemicals

Furfural (F) was obtained from Sigma-Aldrich (USA) and hydroxy methyl furfural (HMF) was obtained from Sigma-Aldrich (China). Orthophosphoric acid was purchased from Merck (Darmstad, Germany), monosodium hydrogen phosphate salt was obtained from Riedel-de Haën (Germany) and acetonitrile was

purchased from Sigma-Aldrich (Israel). Ultra-pure water (18 MΩ) was obtained from Millipore Simplicity device.

5.2. Instrumental

Chromatography analyses were carried out with an Agilent 1260 HPLC device, which consists of a degasser, pump, autosampler, autoinjector, temperature controlled column compartment and a diode array detector (DAD) detector. Analyses were done by using isocratic elution system with a 0.5 mL/min flow rate and the mobile phase A was 50 mM pH 2.5 phosphate buffer and mobile phase B was acetonitrile. During analysis, mobile phase composition was adjusted as 95:5 (MP A: MP B). Separations were carried on an Agilent Poroshell 120 EC-C18 150x3 mm 2.7 µm particle sized column. Agilent Poroshell 120 EC-C18 3x5mm guard column was also used to protect analytical column. Temperature of the column compartment was 25°C and injection volume was 20 µL. Monitoring of the analytes were made by using a DAD detector at 284 nm wavelength.

5.3. Preparation of stock and standard solutions

Stock solutions of F and HMF were prepared as 1000 ppm in acetonitrile. Standard solutions and quality control solutions (QC) of F and HMF were prepared by diluting of these stock solutions with mobile phase. Concentration of standard solutions were 0.1, 0.5, 1, 5, 10 ppm and concentration of the QC samples were 0.8, 3 and 8 ppm for low, medium and high level, respectively.

5.4. Preparation of samples

Honey samples were prepared after a cleaning procedure. For this purpose, 5 g honey sample was weighted and dissolved in 25.0 ml phosphate buffer solution. Than 750 µl Careez I and 750 µl Careez II solutions were added to clean the sample and they were centrifuged during 20 min at 4500 rpm. Supernatant was filtered and inserted in a vial to determine the F and HMF.

5.5. Validation

Developed analytical method was validated according to the USP guideline (30). For this purpose system suitability, accuracy, precision, specifity and robustness and ruggedness of the method were investigated.

5.5.1. System suitability test

Before performing validation experiments, system suitability test (SST) has to be applied to indicate that HPLC system and method are capable of providing data with admissible quality. SST was performed by investigating capacity factor, tailing factor, theoretical plates number, resolution and also relative standard deviation (RSD) of the peak areas. For this purpose, a quality control solution at a concentration of 0.8 ppm was used in six repetitions.

5.5.2. Accuracy and precision

Accuracy and precision of the method were evaluated by two approaches as intra-day and inter-day studies. For this purpose, standard mixtures of each compound at three different concentration values were prepared by diluting stock solution and concentration values were as 0.8, 3 and 8 ppm. Measurements were made as three repetitive of three injections. Recovery value of quality control samples at low, medium and high levels were used for accuracy and relative standard deviation were used for precision calculation.

5.5.3. Specifity

The specifity of the method was evaluated by using spiked honey samples. For this purpose, each standard solution was spiked to same honey sample and chromatograms of these solutions were investigated.

5.5.4. Robustness and ruggedness

Robustness and Ruggedness of the method was performed to realize whether the method was susceptible to variations in method parameters or not. For this purpose, some small changes were applied deliberately on temperature, buffer to acetonitrile ratio and pH parameters and recovery and RSD of the recovery values were recorded.

Acknowledgements: This study was supported by the TÜBİTAK, 215S651 Grant.

Author contributions: Concept – E.T.A., S.H., M.C.; Design – E.T.A., S.H.; Supervision – A.A.; Resources – E.T.A, S.H., M.C; Materials – E.T.A., M.C., A.A.; Data Collection and/or Processing – E.T.A., S.H.; Analysis and/or Interpretation – E.T.A., S.H., M.C.; Literature Search – S.H.; Writing – E.T.A., S.H., M.C., A.A.; Critical Reviews – E.T.A, S.H., M.C., A.A.

Conflict of interest statement: The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Wagner KH, Reichhold S, Koschutnig K, Cheriot S, Billaud C. The potential antimutagenic and antioxidant effects of Maillard reaction products used as "natural antibrowning" agents. Mol Nutr Food Res. 2007; 51 (4): 496–504. [CrossRef]

[2] Mlotkiewicz JA. The role of the Maillard Reaction in the food industry. In: Brien JO, Nursten HE, Crabbe MJC, Ames JM, (Eds). The Maillard Reaction in Foods and Medicine. The Royal Society of Chemistry Special Publication, Cambridge, England, 1998, pp. 19–27.

[3] Ruiz-Matute A, 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): 7317–7322. [CrossRef]

[4] Ulbricht RJ, Northup SJ, Thomas JA. A review of 5-hydroxymethylfurfural (HMF) in parenteral solutions. Fund App Toxicol. 1984; 4: 843–853. [CrossRef]

[5] Bruce WR, Archer MC, Corpet DE, Medline A, Minkin S, Stamp D, Yin Y, Zhang XM. Diet, aberrant crypt foci and colorectal cancer. Mutat Res.1993; 290: 111-118. [CrossRef]

[6] Zhang XM, Chan CC, Stamp D, Minkin S, Archer MC, Bruce WR. Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-furaldehyde in thermolyzed sucrose. J Carcinog. 1993; 14(4): 773-775. [CrossRef]

[7] National Toxicology Program (NTP), Toxicology and carcinogenesis studies of 5-(Hydroxymethyl)-2-furfural (CAS No. 67-47-0) in F344/N rats and B6C3F1 mice (gavage studies). Natl Toxicol Program Tech Rep Ser. 2010; 554:7-13, 15-9, 21-31.

[8] Glatt H, Schneider H, Murkovic M, Monien BH, Meinl W. Hydroxymethyl-substituted furans: mutagenicity in Salmonella typhimurium strains engineered for expression of various human and rodent sulphotransferases. Mutagenesis. 2012; 27 (1): 41-48. [CrossRef]

[9] Monien BH, Engst W, Barknowitz G, Seidel A, Glatt H. Mutagenicity of 5-Hydroxymethylfurfural in V79 Cells Expressing Human SULT1A1: Identification and Mass Spectrometric Quantification of DNA Adducts Formed. Chem Res Toxicol. 2012; 25 (7): 1484–1492. [CrossRef]

[10] International Agency for Research on Cancer (IARC).Dry cleaning, some chlorinated solvents and other industrial chemicals. In. IARC Monographs on the Evaluation of Carcinogenic risks to Humans. 1995; 63: 409-429.

[11] Islam MN, Khalil MI, Islam MA, Gan SH. Toxic compounds in honey. J Appl Toxicol. 2014; 34(7): 733-42. [CrossRef]

[12] Nagy S, Dinsmore HL. Relationship of furfural to temperature abuse and flavor change in commercially canned single-strength orange juice. J Food Sci. 1974; 39: 1116–1119. [CrossRef]

[13] Jeuring HJ, Kuppers FJ. High performance liquid chromatography of furfural and hydroxymethylfurfural in spirits and honey. J Assoc Off Anal Chem. 1980; 63, 1215–1218.

[14] Truzzi C, Annibaldi A, Illuminati S, Finale C, Rossetti M, Scarponi G. Determination of very low levels of 5-(hydroxymethyl)-2-furaldehyde (HMF) in natural honey: comparison between the HPLC technique and the spectrophotometric white method. J Food Sci. 2012; 77(7): 784-90. [CrossRef]

[15] Langeveld van HEAM, Goossens PMH. Gas chromatographic determination of 5-hydroxymethylfurfural (HMF) in honey. Food and Agriculture Organization 1981. http://agris.fao.org/agris-search/search.do?recordID=XE8182776 (accessed January 11, 2018).

[16] Teixidó E, Santos FJ, Puignou L, Galceran MT. Analysis of 5-hydroxymethylfurfural in foods by gas chromatography–mass spectrometry. J Chromatogr A. 2006; 1135 (1): 85–90. [CrossRef]

[17] Bahaffi SO, Al-Lihaibi SS. Determination of volatile organic compounds in local honey by gas chromatography-mass spectrometer. Commun Fac Sci Univ Ank Series B. 2005; 51(2): 1-12. [CrossRef]

[18] Chernetsova ES, Revelsky IA, Morlock GE. Fast quantitation of 5-hydroxymethylfurfural in honey using planar chromatography. Anal Bioanal Chem. 2011; 401(1): 325-332. [CrossRef]

[19] Morales FJ, Jimenez-Perez S. Hydroxymethylfurfural determination in infant milk based formulas by micellar electrikinetic capillary chromatography. Food Chem. 2001; 72: 525-531. [CrossRef]

[20] Zappala M, Fallico B, Arena E, Verzera A. Methods for the determination of HMF in honey: a comparison. Food Control. 2005; 16: 273-277. [CrossRef]

[21] Rufián-Henares JA, Garcia-Villanova B, Guerra- Hernandez E. Determination of furfural compounds in enteral formula. J Liq Chrom Rel Technol. 2001; 24(19): 3049-3061. [CrossRef]

[22] Blanco Gomis D, Gutiérrez Alvarez MD, Sopeña Naredo L, Mangas Alonso JJ. High-performance liquid chromatographic determination of furfural and hydroxymethylfurfural in apple juices and concentrates. Chromatographia. 1991; 32: 45-48. [CrossRef]

[23] 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. [CrossRef]

[24] Spano N, Casula L, Panzanelli A, Pilo MI, Piu PC, Scanu R, Tapparo A, Sanna G.An RP-HPLC determination of 5-hydroxymethylfurfural in honey: The case of strawberry tree honey. Talanta. 2006; 68(4): 1390-1395. [CrossRef]

[25] Zappala M, Fallico B, Arena E, Verzera A. Methods for the determination of HMF in honey: a comparison. Food Control. 2005; 16(3): 273-277. [CrossRef]

[26] Codex Alimentarius, Food and Agriculture Organization of the United Nations. 1981. http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCODEX%2BSTAN%2B12-1981%252Fcxs_012e.pdf, (accessed January 11, 2018).

[27] Codex Alimentarius, Alinorm 01/25. Draft Revised Standard for Honey at Step 8 of the Codex Procedure; EU Directive /1/110/2001 of 02/12/2001 (L 10/47).

[28] EU Risk Assessment Report-Furfural. Summary Risk Assessment Report. 2008. https://echa.europa.eu/documents/10162/8b8d48c6-b5ea-459f-98d0-4b8521b97e7c (accessed January 11, 2018).

[29] Lachenmeier DW, Rehm J. Comparative risk assessment of alcohol, tobacco, cannabis and other illicit drugs using the margin of exposure approach. Sci Rep. 2015; 5: 1-7. [CrossRef]

[30] United States Pharmacopeia (2014) United States Pharmacopeial Convention. United States Pharmacopeia, Rockville.