



Determination of Folic Acid by Ultra-High Performance Liquid Chromatography in Certain Malt-based Beverages after Solid-Phase Extraction

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Received: 13 December 2016

Accepted: 29 June 2017

DOI: 10.18466/cbayarfbe.339315

Abstract

The aim of the present work was to develop and validate an efficient Ultra-High Performance Liquid Chromatography (UHPLC) method for the determination of folic acid (FA) in malt-based beverages. Solid-phase extraction (SPE) procedure was used for cleanup and preconcentration of the malt-based beverages before the UHPLC analysis. The analysis was performed in a C18 column (2.1x50 mmx1.8 μm) using a solvent system of ACN: 0.1 % formic acid in water (10:90, v/v) by isocratic elution. Injection volume was 5 μL . The flow rates of the mobile phase were maintained at 0.2 mL min^{-1} for 0.00–4.00 min and 0.5 mL min^{-1} for 4.01–12.00 min. Methyl paraben was used as the internal standard (IS). The FA and IS signals were detected at 284 nm and 254 nm, respectively. Under these conditions, FA and IS were separated in 3.6 min and 11.4 min, respectively. The method was successfully validated in terms of precision, accuracy, linearity, limits of detection (LOD) and quantification (LOQ) parameters. The relative standard deviations for intra- and inter-day precision were less than 1.5%. Good linearity with a high correlation coefficient was achieved over the concentration range of 20.13 $\mu\text{g L}^{-1}$ – 2004 $\mu\text{g mL}^{-1}$ for FA. The LOD and LOQ values were 6.66 $\mu\text{g L}^{-1}$ and 20.13 $\mu\text{g L}^{-1}$, respectively. Good recovery values were found ranged between 99.1% and 106% for boza and vitamin fortified malt drink. The proposed method was successfully applied for the determination of FA in malt beers, vitamin fortified malt drinks and boza samples.

Keywords: Boza, Folic acid, Malt-based beverages, Method development, UHPLC, Validation.

1. Introduction

Folic acid (FA) stands out as a molecule having biological importance in recent years. The name folate usually outlines a class of compounds with chemical structures related to pteroylmonoglutamic acid and generally recognised as folic acid (FA, vitamin M, B9 or B11) [1, 2]. There are more than 100 compounds that can be defined as folates [3] and folic acid is the simplest synthetic and oxidised form of folates [4]. Folate is present in legumes, egg, kidney, liver, tongue, citrus fruits, leafy green vegetables, beans, wheat germ, and yeast [1, 5-8].

Folate is synthesized solely by microorganisms and plants so that humans need to intake the vitamin from different dietary sources as a natural form or from supplements and enriched foods as a folic acid [5, 6]. Since FA is more stable, cheap and easily absorbable synthetic form and it reveals higher bioavailability than naturally occurring food folate, this water soluble B group vitamin is increasingly utilized for food

fortification purposes [9]. The recommend daily intakes (RDI) for folate change from 150 to 600 μg per day depending on the age and sex of the individuals and also vary notably from country to country [8]. The United States Public Health Service suggests that all women of childbearing age should use 400 μg daily dose of folic acid for the prevention of spina bifida or other neural tube defects [10]. Currently, some European countries recommend that intake 500 μg of daily folic acid for women who are breastfeeding [8].

When FA is converted into folates in the body, it acts an important role in various biochemical pathways including the synthesis of red blood cells, amino acid metabolism such as methionine and homocysteine, nucleotide synthesis and formation of RNA and DNA, DNA replication, repair and methylation, development of tissues and the brain of the foetus and the growth of a baby [1, 8, 11, 12]. FA deficiency has been related to some critical diseases for instance gigantocytic anemia and leukopenia, devolution of mentality, Alzheimer's

disease, psychosis, neural tube defects (NTDs), certain forms of cancer such as pancreatic, colorectal and breast cancers [8, 11, 13, 14]. Furthermore, blood homocysteine concentration increases due to the inadequate intake of FA which may lead to coronary heart disease and stroke [14]. Briefly the vitamin is vital for humans for growth, reproduction and continue of normal body function and so that FA shows a critical role in the human nutrition [12].

When the increasing significance of folates in health or disease take into consideration, quantitative analyses of FA in foods is very important [15]. Therefore, analysis of FA with a reliable analytical method is very important. For this purpose numerous analytical methods have been used in the literature such as microbiological assay [16], immunoassay [17], UV spectrophotometric [18], high-performance liquid chromatography (HPLC) [9, 19, 20, 21], liquid chromatography-mass spectrometry (LC-MS) [22], capillary electrophoresis (CE) [3, 5, 9, 13], ultra-high performance liquid chromatography (UHPLC) [1] and UHPLC-MS [23].

The microbiological assay is usually used as a standard method for determination of food folates. Although it has high sensitivity, this method cannot separate the various forms of folate. Furthermore, the experimental method is time-consuming and tedious. On the other hand, HPLC is more accurate, easy and can determine individual folate forms when compared to this method [17, 19]. Although LC-MS provide many advantages, this instrument is quite expensive and necessitate expertise of the analyst [24]. Among these techniques, HPLC is commonly used for FA analysis in different food matrices with various separation columns, mobile phases, and detectors however there is still need for the optimisation and development of novel analytical methods [15].

The Ultra-high performance liquid chromatography (UHPLC) has emerged as a powerful method widely used nowadays due to using new technologies small (sub-2- μm) particle size packed columns with small diameter. UHPLC has recently been started popular in various analyses because it has significant advantages than the HPLC technique such as its higher speed and efficiency, less time and solvent consumption criteria by keeping the equivalent separation power [25-27]. Furthermore, analytes can be detected at very low concentrations by the UHPLC system due to improved signal-to-noise ratio [28].

There are some standard official methods which are based on microbiological assay to measure total folates in vitamin preparations [29] and in certain foods such as fortified infant formula [30], cereals [31], and for foodstuffs [32, 33]. However there are no standard methods enacted yet for folates in foods with chromatographic methods. Because folate

determinations show large differences for various foods with high relative standard deviation (RSD) values [24, 33]. So that possibility of matrix specific problems must be get through for different samples from the investigators [15].

Malt beverages, which are fermented drinks, are commonly known as the healthy drinks. Moreover these drinks are FA source for pregnant population and also are believed to help increase milk production during the lactation. Since the breast milk is a source of folic acid for the breastfed infant, malt beverages including alcohol free vitamin fortified malt drink and boza are selected for investigation of folic acid in this research [34]. Boza is a Turkish traditional malt beverage which is made from millet, maize, wheat or rice and their flours by fermentation. The highly viscous beverage consumed especially in winter is quite nutritious due to its vitamin content, lactic acid, protein, fibre and carbohydrate [35, 36].

The objective of present study is to investigate the FA level in certain malt-based beverages (malt beers, boza and vitamin fortified malt drinks) by a reliable UHPLC method. To the best of author's knowledge there are limited study about malt beverages by UHPLC method and also there is no report in the literature about FA analysis in boza by the method. In this study a simple, sensitive and efficient UHPLC method was developed and validated for the determination of FA in malt-based beverages which are consumed in Turkey.

2. Experimental

2.1 Chemicals and Materials

Folic acid (FA), methyl paraben (IS), sodium acetate ($\geq 99.0\%$), sodium L-ascorbate (99.0%), acetonitrile, methanol, formic acid and sodium phosphate dibasic dihydrate ($\geq 98.5\%$) were provided from Sigma (St. Louis, MO, USA). Glacial acetic acid (99.5%) was purchased from Carlo Erba (Milan, Italy). Orthophosphoric acid (Puriss 85.0%) was obtained from Riedel-de Haen (Seelze, Germany). Supelclean 500 mg LC-18 SPE cartridges (Supelco, Bellefonte, PA, USA) were utilized for solid phase extraction (SPE). Analytical reagent grade chemicals were used in all the experiments. All of the malt beverages samples (malt beers, boza and vitamin fortified malt drink) were purchased from markets in Eskişehir (Turkey) and were analysed on the same day.

The ultra-pure deionized water was purified by the Millipore, Synergy Water Purification System (Rotterdam, Netherland). All the solutions were degassed with a Sonorex ultrasonic bath (Bandelin, Berlin, Germany). The samples were dissolved and mixed by a Vortex (2G 560-E, Daigger Scientific Industries, Bohemia, USA). SevenCompact pH/Ion meter (Mettler Toledo, Columbus, Ohio, USA) was utilized for

measuring the pH of solutions. Evaporation procedures were achieved by using a rotary evaporator (Rotavapor R-200, heating Bath B-490, Buchi, Flawil, Switzerland).

2.2 Preparation of Standard Solutions

Standard stock solution of folic acid was prepared in 0.1 M phosphate buffer (pH 7.0) containing 1.0% (w/v) sodium ascorbate at a concentration of 2.27×10^{-3} M [37]. The pH of the solution was adjusted by orthophosphoric acid. The stock FA solution further diluted with 0.01 M acetate (pH 4.9) for preparation of 2.27×10^{-5} M and 6.81×10^{-6} M FA solutions. 6.81×10^{-6} M of FA solution used for the preparation of calibration standard solutions. The appropriate amount of methyl paraben (IS) was weighed for preparation of 7.62×10^{-3} M stock solution in methanol. Then 4.57×10^{-5} M of IS was prepared from this solution by diluting with water and was used further experiments. All the calibration standard solutions were prepared immediately before use by dilution with water. A 20 mM sodium acetate buffer was prepared by dissolving sodium acetate in water and pH of the solution was adjusted to 4.0 by using acetic acid. Stock solutions were divided to aliquots and stored in the -20 °C. All working standard solutions were stored in the dark at 4 °C.

2.3 UHPLC Equipment and Conditions

An Agilent 1290 Infinity UHPLC system (Waldbronn, Germany) equipped with a UV detector was used in this study. An Eclipse Plus RRHD C18 column (2.1x50 mmx1.8 μ m) and guard column (2.1x5 mmx1.8 μ m) from Agilent (Waldbronn, Germany) were utilized in the UHPLC analyses. The isocratic elution was used and the mobile phase consisted of 10% acetonitrile and 90% water with 0.1% formic acid. The flow rates of the mobile phase were maintained at 0.2 mL min⁻¹ for 0—4 min and 0.5 mL min⁻¹ for 4.01—12 min. The signals were detected at 284 nm within the ten minute and the wavelength was changed to 254 nm at ten minute. 284 nm and 254 nm are the maximum absorbance wavelengths of FA and IS, respectively. Injection volume was 5 μ L. Methyl paraben was used as an internal standard (IS). The UHPLC column was kept at 25 °C temperature. Oto sampler was cooled to 10 °C during the experiments.

2.4 Sample Preparation

2.4.1 Preparation of Samples to Solid-Phase Extraction (SPE)

Firstly all of the gases in the beer samples were removed by using ultrasonic bath for a while. 100% malt beer, vitamin fortified malt beverage and boza samples were filtered from 0.45 μ m syringe filter prior to SPE procedure. Boza samples are considerably viscous than the malt beverages so that they require different sample pre-treatment explained as following. Approximately 25–30 g boza sample was weighed and it was centrifuged for 30 min at 27,216 g. 10 mL of supernatant was

transferred to a tube and 20 mL of acetonitrile was added for the precipitation of its proteins. And then clear phase was evaporated to dryness with rotavapor [38]. Residue was dissolved in 10 mL of water, filtered from 0.45 μ m and then passed through the SPE column.

2.4.2 Solid-Phase Extraction (SPE) Procedure

The SPE process was succeeded by a previous method that reported elsewhere [39]. Firstly, C18 SPE column was conditioned by washing with methanol (5 mL), and water (10 mL) without allowing the cartridge to dry out. 10 mL of malt beverage sample was passed from the cartridge. Then, folic acid was eluted through 3 mL of methanol:sodium acetate buffer (20 mM, pH 4) (50:50, v/v) and this solution was homogenised with vortex agitation. 0.5 mL of methyl paraben (IS) (4.57×10^{-5} M) was added to the 1.0 mL of the eluate and the volume was then completed to 4 mL by methanol:sodium acetate buffer (20 mM, pH 4). Other dilutions of the samples are also adjusted to as 1:8 (V:V, IS:Total solution) ratio. After mixing, the sample solution was injected to the UHPLC column.

2.5 Statistics

GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA) was used for the statistical analyses of the experimental data.

3. Results and Discussion

3.1 Optimisation of UHPLC Method

The ideal chromatographic conditions were optimised by using parameters including flow rate, injection volume, mobile phase composition and detection wavelength for FA along with IS. Different mobile phase systems of isocratic or gradient elution and different proportions of acetonitrile and 0.1% formic acid in water were tried for determine to best chromatographic conditions. Thus FA and IS were successfully separated from other peaks in food matrices. However it was probable to obtain the peaks in a shorter time but a poor resolution was seen when the samples were analysed. Methyl paraben was a most suitable compound as an IS for malt beverages analyses with the UHPLC system. Final concentration of IS was used as 5.71×10^{-6} M at all of experiments. Folic acid and IS were separated in nearly 3.6 min and 11.4 min, respectively in the optimum UHPLC conditions (Figure 1) which was mentioned in the 'Experimental Section 2.3'.

The compounds are found at quite low concentration levels in food samples so that a preconcentration and clean-up step is necessary [26]. SPE not only provides preconcentration of analyte species but also separates matrix components which are potential interfering. Accordingly SPE method was used for this purpose in this study. Since the extraction procedure was a very important step for FA determination, various SPE methods by using strong anion exchange (SAX) and

reverse phase C18 SPE columns were tried for the extraction of FA from malt beverages. Besides different solutions were also tested to get optimum extraction conditions for C18 SPE column. The best performance was achieved with C18 SPE column and the SPE procedure was given in the ‘Experimental Section 2.4.2’

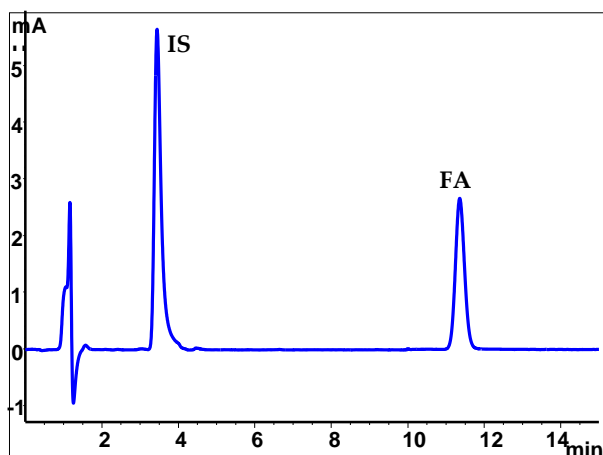


Figure 1. Typical UHPLC chromatogram of standard FA (2.27×10^{-6} M) and IS (5.71×10^{-6} M) in optimum conditions

3.2 Validation of the UHPLC Method

The International Conference on Harmonization (ICH) of Technical Requirements (CPMP/ICH/281/95) guidelines was performed for the validation of the method with following validation parameters: precision, linearity, accuracy, limit of detection (LOD), and limit of quantification (LOQ) [40].

3.2.1 Precision

The precision of the method was assessed by intra-day and inter-day repeatability for three different concentrations of FA. For this purpose 9.08×10^{-7} M, 2.27×10^{-6} M and 4.54×10^{-6} M of FA solutions with fixed amount of 5.71×10^{-6} M IS were used in the repeatability study. These were injected six times for three different days. The peak normalization (PN) values were calculated by dividing the area values by their retention times. After, the rate of peak normalizations (PN) were obtained by dividing those of PN values of FA to IS. The intra-day (n=6 each) and inter-day (n=18) results of rates of peak normalization and their statistical parameters are presented in Table 1. The RSD% values change between 0.20–0.69 (intra-day) and 0.52–1.37 (inter-day), and these results express that the method is highly repeatable.

Table 1. The results of repeatability obtained from determination of FA using ratios of peak-normalization values ($R = PN_{FA}/PN_{IS}$)

9.08×10^{-7} M FA	Intra-day results (n=6 each)			Inter-day results (n=18)
	First Day	Second Day	Third Day	
\bar{X}	1.99	1.98	2.00	1.99
SD	0.01	0.01	0.01	0.01
RSD%	0.69	0.45	0.25	0.52
2.27×10^{-6} M FA	Intra-day results (n=6 each)			Inter-day results (n=18)
	First Day	Second Day	Third Day	
\bar{X}	4.96	5.00	4.89	4.95
SD	0.01	0.01	0.02	0.05
RSD%	0.23	0.21	0.31	1.00
4.54×10^{-6} M FA	Intra-day results (n=6 each)			Inter-day results (n=18)
	First Day	Second Day	Third Day	
\bar{X}	8.62	8.38	8.61	8.53
SD	0.02	0.03	0.02	0.12
RSD%	0.20	0.40	0.20	1.37

\bar{X} : mean; SD: standard deviation; RSD%: percent of relative standard deviation; CL: confidence limit for the 95% probability level

3.2.2 Linearity and Sensitivity

Calibration curve was found (concentrations against RPN values) by preparing six different concentrations of the standard FA solutions with fixed amount of 5.71×10^{-6} M IS as seven replicates. The method is linear in the calibration range of $20.13 \mu\text{g L}^{-1}$ (1.51×10^{-8} M) – $2004 \mu\text{g L}^{-1}$ (4.54×10^{-6} M) of FA with a high correlation coefficient ($r^2=0.9998$) and the intercept of the curve is not considerably different from zero. The LOD and LOQ values are $6.66 \mu\text{g L}^{-1}$ and $20.13 \mu\text{g L}^{-1}$, respectively. Results are summarised in Table 2.

Table 2. Calibration results of FA with their statistical analysis under the optimum UHPLC conditions (n=7)

Linearity range ($\mu\text{g L}^{-1}$)	20.13–2004
Slope\pmSD	$(24360 \pm 51.37) \times 10^2$
Intercept\pmSD	-0.0649 ± 0.0111
r^2	0.9998
$S_{y,x}$	0.0514
LOD ($\mu\text{g L}^{-1}$)	6.66
LOQ ($\mu\text{g L}^{-1}$)	20.13

r: correlation coefficient; $S_{y,x}$: standard deviation of calibration equation; LOD: limit of detection; LOQ: limit of quantification; n: number of experiments

3.2.3 Accuracy

Accuracy was tested by means of recovery. Boza and vitamin fortified malt drink samples containing known amounts of folic acid were spiked with three addition levels of standard folic acid for determination of recovery. For this purpose, boza samples spiked with 4.54×10^{-7} , 9.08×10^{-7} and 2.27×10^{-6} M of folic acid and vitamin fortified malt drink samples spiked with 9.08×10^{-7} , 2.27×10^{-6} and 4.54×10^{-6} M of folic acid were used in the recovery experiments. This spiking procedure was repeated twice for all concentrations. Two unspiked samples for each boza and vitamin fortified malt drink were also used in this experiments. Three measurements were achieved for each level of addition. As seen in Table 3, good recovery values were found ranged between 97.8% and 106% for boza and vitamin fortified malt drink. These recovery values are acceptable for quantification of FA in boza and malt-based beverages.

3.2.4 Stability

The stability of the various folates changes and depends on oxidants, pH and catalysts, even the type of buffer ions effect the vitamins [33]. For this purpose, it was mentioned in Experimental section (2.2), sodium ascorbate was added to increase the stability of the FA solution [37]. Furthermore, FA is sensitive to degradation by heat, oxidation, and ultraviolet light [41]. Therefore, all of the FA solutions were either freshly prepared or they were kept in a refrigerator in the dark during analysis.

The stability of FA was assessed under different storage conditions at three concentrations (2.83×10^{-7} , 9.08×10^{-7} and 2.27×10^{-6} M). For this purpose, short-term, long-term and refrigerator stabilities of FA were studied. To test short-term stability, solutions were preserved at room temperature for 24 h. The long-term stability was

evaluated after storage of FA solutions in a freezer at -20 °C for 24 days. For refrigerator stability, the solutions were stored at 4 °C for 7 days and then analyzed. The stability results were considered by comparing peak area ratios of the analytes with those of freshly prepared samples. The results are given in Table 4 show that FA display satisfactory stability in aqueous solutions at these conditions except long-term stability. FA was started to deteriorate during storage at -20 °C for 24 days (Long-term stability). In refrigerator conditions, FA was stable up to 7 days of storage when the solutions were protected from light.

Table 3. Concentration, recovery, standard and relative standard deviation values of FA in boza and vitamin fortified malt drink samples determined by UHPLC (n=2^a; l=3)

Spiked FA concentration (M)	Recovery(%) ^b (n=2; l=3)	SD	RSD %
boza			
4.54×10^{-7}	104.40	5.85	5.60
9.08×10^{-7}	99.12	4.68	4.72
2.27×10^{-6}	106.37	9.01	8.47
vitamin fortified malt drink			
9.08×10^{-7}	99.79	3.26	3.27
2.27×10^{-6}	105.62	1.54	1.45
4.54×10^{-6}	97.83	3.58	3.65

^a n: number of experiments; l: number of injections

^b Recovery= [(spiked sample result - unspiked sample result)/(known spiked concentration)×100]

Table 4. Stability of FA under different conditions (n = 3)

Added FA (mol L ⁻¹)	Short-term stability (24 h, room temperature)		Long-term stability (24 days, -20 °C)		Refrigerator stability (7 days)		
	Remained (%) (mean±SD)	RSD (%)	Remained (%) (mean±SD)	RSD (%)	Remained (%) (mean±SD)	RSD (%)	
FA	2.83×10^{-7}	103.36±13.52	13.07	104.05±0.77	0.74	91.76±3.82	4.17
	9.08×10^{-7}	107.02±2.60	2.43	150.78±3.48	2.31	94.93±2.44	2.57
	2.27×10^{-6}	114.98±0.54	0.47	121.64±0.91	1.37	96.78±0.84	0.75

Remained % = (peak normalisation of FA after the mentioned storage conditions/peak normalisation of fresh FA)×100

3.2.5 Robustness

The robustness of the proposed method was studied by doing deliberate alterations in flow rate of mobile phase, column temperature, composition of mobile phase and detector wavelength. Parameters regarding robustness are given in Table 5. The RSD% values are smaller than 6.0 and it can be considered that the method is highly robust.

Table 5. The results of robustness of FA (n= 3)

Parameter	RSD%	SE
Flow rate of solvent (0.15 mL min ⁻¹)	3.63	0.04
Flow rate of solvent (0.3 mL min ⁻¹)	2.05	0.03

Column temperature (25°C)	0.65	0.01
Column temperature (30°C)	0.26	0.01
The composition of mobile phase (9%)	5.97	0.05
The composition of mobile phase (11%)	3.14	0.06
Detector wavelength (280 nm for FA)	5.32	0.10
Detector wavelength (284 nm for FA)	1.42	0.03

$$SE \text{ (standard error of mean)} = \frac{SD}{\sqrt{n}}; SD: \text{standard deviation}$$

3.3 Application of the UHPLC Method

The proposed method was applied to 7 samples of malt-based beverages. FA amounts in malt-based beverages were calculated via equation obtained from calibration curve and the results are given in Table 6. As it can be seen from the Table 6, the contents of FA were found as 36.63–196.80 $\mu\text{g kg}^{-1}$ with good RSD% values in the mentioned malt beverages. FA was not detected in 2 of the boza samples. A typical chromatograms of boza, vitamin fortified malt beverage and malt beer are shown in Figure 2.

Table 6. Results for the determination of FA in the different food samples ($\mu\text{g kg}^{-1}$) (n=6)

Sample	\bar{X}	SD	RSD%
Sample 1 (Boza)	36.63	1.97	5.38
Sample 2 (Boza)	nd*	—	—
Sample 3 (Boza)	nd*	—	—
Sample 4 (Malt Beer)	196.80	2.37	1.20
Sample 5 (Malt Beer)	146.42	1.59	1.09
Sample 6 (Vitamin Fortified Malt Beverage)	36.65	0.92	2.50
Sample 7 (Vitamin Fortified Malt Beverage)	42.96	1.67	3.88

*nd: not detected.

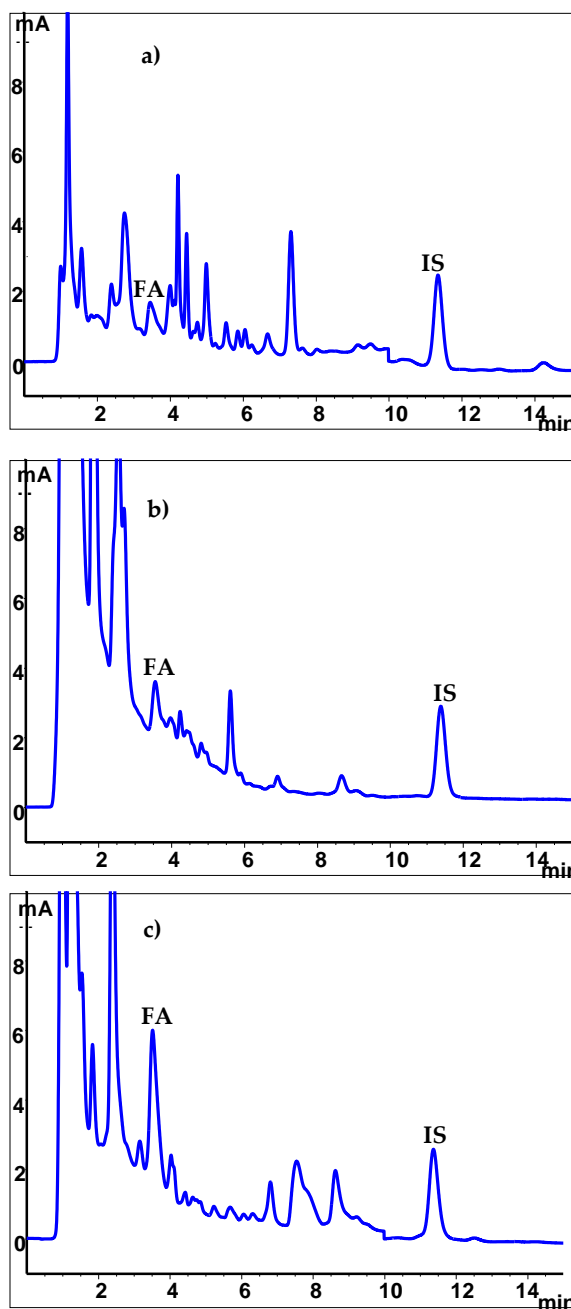


Figure 2. Representative chromatograms obtained from malt-based beverages using the proposed UHPLC method a) Boza, b) Vitamin Fortified Malt Beverage, and c) Malt beer sample.

4. Conclusion

An efficient UHPLC method was developed and validated for the determination of FA in certain malt-based beverages in this study. The validated study reveals that this method is precise, accurate and sensitive. In the current study, FA is separated in a relatively short time by the developed UHPLC method. Besides, UHPLC reduces flow rates of the mobile phase when compared with HPLC and shows an important gain in analysis time and solvent consumption. It is the first study which includes the determination of FA in boza sample. A

comparison of the proposed method with literature was presented in Table 7. Consequently, this work gives a new information about folic acid content in malt-based

beverages, which should be favourable for food databases and the developed method is suitable for the routine quantification of FA in food analysis.

Table 7. Comparison of the developed method with literature for FA analysis

Retention Time for FA (min)	Preconcentration method	Determination method	Linear Range ($\mu\text{g L}^{-1}$)	LOD ($\mu\text{g L}^{-1}$)	Applications	Reference
2.0	—*	UHPLC-DAD	600–12 000	NA	Pharmaceutical preparations	[1]
22	—*	CE	5297–21187	270	Lentils	[3]
3.5 (Approx.)	Complexation with Fe(III) and CPE	HPLC-DAD	20–1200	6.06	Food samples	[21]
2.8	—*	UHPLC-MS-MS	0.01–0.500	0.05	Serum	[23]
—	UAE	UV spectrophotometry	0.6–180	0.19	Foods and drugs	[42]
11 (Approx.)	C18 SPE	HPLC-DAD	500–20 000	100	Tarhana	[43]
15	Trienzyme extraction procedure	HPLC-coulometric electrochemical detection	50–2500	1.3	Fortified fruit juices and cereal products	[44]
3.6	C18 SPE	UHPLC-UV	20.13–2004	6.66	Malt-based beverages (boza, beer, malt drinks)	This Study

NA Not available, CE Capillary electrophoresis, CPE cloud point extraction, UAE ultrasonic-assisted extraction, SPE solid-phase extraction, * the method includes sample preparation and purification procedures which are including dissolution or protein precipitation.

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