Asian Journal of Chemistry Vol. 22, No. 10 (2010), 7997-8001

Determination of Folic Acid in Syrian Cereals by High Performance Liquid Chromatography with UV-DAD Detection After SPE Extraction

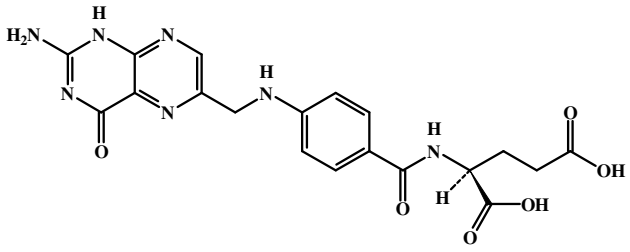
SAAD ANTAKLI*, NAZERA SARKIS† and NAYLA NABO
Department of Chemistry, Faculty of Science, University of Aleppo, Aleppo, Syria
E-mail: antakli@scs-net.org

Folic acid is a water soluble vitamin which has been determined in some Syrian cereals such as (flour, rice, semolina, corn, gram, borghol, lentil, triticum durm, triticum aestivum, lima bean) by HPLC-DAD detection after solid phase extraction clean up. For analytical conditions, we studied the folic acid determination by C_8 and ODS columns. Isocratic system was used for C_8 column. The mobile phase was with a volumetric mixture of 92 % A and 8 % B. The second one for ODS column, the separation was in a gradient system. The flow rate for both columns were 1 mL/min, the wavelength was 280 nm. The concentrations of folic acid varied from 0.02-1.90 μg/g.

Key Words: Folic acid, SPE, Comparison chromatographic columns.

INTRODUCTION

Folic acid (often called "folate" or folacin) has been investigated in 1920, it takes its name from Latin word folium, meaning Leaf, it's a water soluble vitamin. It is also called 'vitamin B_9'. The chemical structure (**I**) of folic acid determined by Bob Stokstad in 1943.



Structrue of folic acid (m.f. $C_{19}H_{19}N_7O_6$) (**I**)

Folic acid prevents from hazards resulting from oxidative stress or disorders of cell division and DNA repair, also prevents neural tube defects, Alzheimer's disease and colon cancer. To prevent these defects adequate folate should be given to the women in the first month of their pregnancy[1].

†Department of Food & Analytical Chemistry, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria.

In 1997 solid phase extraction (SPE) was tested as pre-separation techniques for HPLC determination. The choice of the sorbent, solvents and washing is important. There are two techniques in solid phase extraction, the first one is to pulled sample through the sorbent and the second one is to pushed the sample. To activate the SPE cartridge, it must washed by HPLC grade methanol then with ultrapure deionized water[2,3].

Folic acid has been determined in some pharmaceutical products and food by various method such as titration, but this method required tedious treatments of samples and takes a long time as well as it is not accusers[4], GC techniques[5]. The folic acid has also been determined by HPLC with many mechanism like ion exchange chromatography[6] and ion pair chromatography[7]. HPLC with many detection types have been used to separate and determinate folic acid such as MS detector[8,9], flourecense[10], IR[11], electrochemical detection[12] and UV detection[13,14]. Also vitamin B_9 separated and determined on many stationary phases such as amide[15], C_8 and C_{18} columns[16].

EXPERIMENTAL

The chromatograms were obtained by using Hitachi liquid chromatography equipped with a diode array detector (DAD) Hitachi L-2200 pump Hitachi L-2130 column oven Hitachi L-2350 and auto sampler Hitachi L-2200. The columns were from MN company. ESP was from MN company (Germany), centrifuge model 90-1 Shanghai surgical instruments factory (China) Altrasonic 405 from Hwashin Technology (Korea), Micropipt IsoLap (Germany).

Standard vitamin (B_9) was purchased from Rosh (Switzerland), triethanolamine (TEA), HPLC grade acetonitrile, water, ammonium acetate were purchased from Merck (Germany), methanol HPLC was purchased from ACROS (USA).

Standard preparation: Standard stock solution of folic acid in concentration of (0.2 mg/mL) was prepared by dissolving a required amount of folic acid in diluted solutions. The working standard solutions (0.033-20.000 μg/mL) were prepared by diluting the standard stock solution with diluted solutions.

Calibration curve: To construct the calibration curve, five replications of (25 μL) for standard solution were injected immediately after preparation into C_{18} and C_8 columns and peak areas of chromatograms were measured at 280 nm as it is shown in Fig. 1.

Sample preparation: Syrian cereal samples were taken from many locations. An amount of samples were taken and finely grounded, then 5 g of each samples have been taken to 50 mL volumetric flask and the volume reached by diluted solution to the obtained volume. After putting the flask to shaker for 40 min, the solution has been centrifuged for 10 min then solid phase extraction extraction is employed. To activate the solid phase extraction cartridge, 2 mL of HPLC grade methanol was dispensed into upper part of the cartridge, opened the valve to draw the methanol slowly through the solid phase extraction cartridge, close the valve.

Then 2 mL of water was dispensed into upper part of the cartridge, opened the valve to draw the water slowly through the SPE cartridge, closing the valve as soon as the liquid meniscus is touching the upper frit.

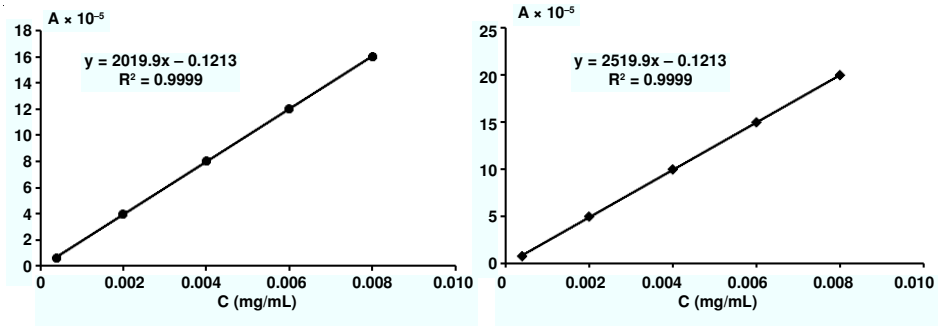


Fig. 1. Calibration curves of folic acid (vitamin B_9) on C_8 column and C_{18} column, respectively

Loading the sample onto the cartridge. Accurately 3 mL of sample was dispensed into upper part of the cartridge by pipette, open the port valve to draw the sample slowly through the cartridge. The same method was repeated for all the samples. Finally 25 µL of each SPE extracted sample was injected into the column and data were recorded. The concentrations of folic acid in the samples were then calculated using peak data and standard curves. The chromatographic conditions for the separation of folic acid in different samples are given in Table-1.

TABLE-1
CHROMATOGRAPHIC CONDITION TO SEPARATE AND DETERMINATE FOLIC ACID IN SOME SYRIAN CEREALS

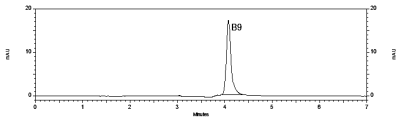
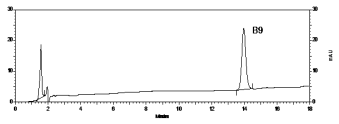
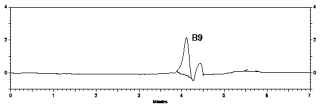
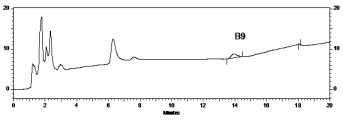
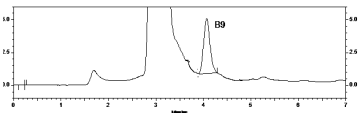
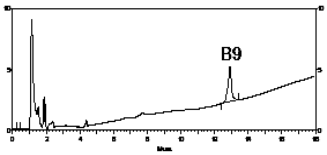
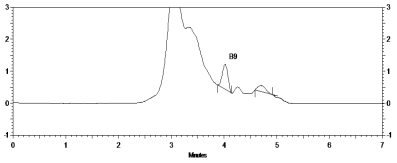
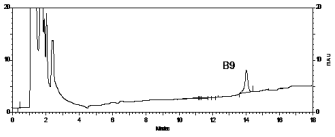
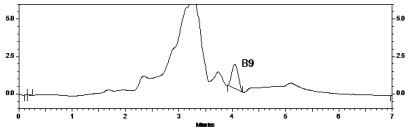
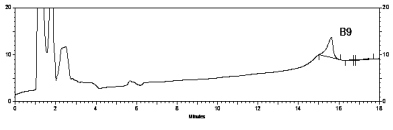
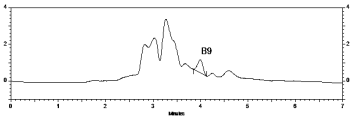
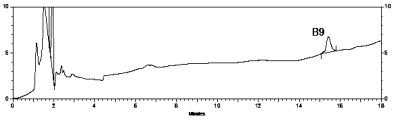
	(Gradient) C_{18} (250 × 4.6)			(Isocratic) C_8 (250 × 4.6)
Mobile phase	A: Water:methanol:acetic acid:TEA) (90:9:1:0.12)			A: Ammonium acetate 0.15 M: methanol (90:10)
	B: Water:methanol:acetic acid:TEA) (27:72:1:0.12)			B: Ammonium acetate 0.15 M: acetonitrile (40:60)
	Time (min)	A	B	
	0	90	10	A = 92 %, B = 8 %
	21	27	73	
Flow rate mL/min	1			1
Wavelength	280 nm			280 nm
Diluted solution	Ammonium acetate 1 M			Ammonium acetate 1 M
Injection volume	25 µL			25 µL

RESULTS AND DISCUSSION

Under the chromatographic conditions employed the topical representative chromatograms of folic acid obtained on C_8 and C_{18} column are shown in Table-2.

We present the results obtained of folic acid in some Syrian cereal samples in Table-3 for each C_8 and ODS columns.

TABLE-2
CHROMATOGRAMS OF FOLIC ACID IN SOME SYRIAN SEREALS ON C_8 AND C_{18} COLUMNS

	Chromatograms of B_9 on C_8	Chromatograms of B_9 on C_{18}
Standard		
Corn		
Wheat flour		
Lentil black		
Rice		
Gram		

Conclusion

In this work, a new chromatographic conditions is developed to determine folic acid in some Syrian cereal samples (flour, rice, semolina, corn, gram, borghol, lentil, triticum durm, triticum aestivum, lima bean). Under the suggested chromatographic conditions the folic acid has been separated and determined on C_8, ODS columns, with a comparable results. The comparison study of separation and determination folic acid on two C_8, ODS columns, give us two choice of a suitable column concerning the analysis purpose, time of analysis, or the products. From the above result it has been found that the shortest time of analysis was on C_8 column about 4 min while C_{18} about 15 min. But it was clear that the separation of folic acid on ODS column was better than on C_8 column. Both column gave a good separation and relatively fine peaks in short time. So we can save lot of solvents, chemical regents and time of analysis.

TABLE-3
AMOUNT OF FOLIC ACID IN SOME SYRIAN CEREALS SEPARATED AND DETERMIEND ON C_8 AND C_{18} COLUMNS

Samples	Vit. B_9 (μg/g) C_8 column	RSD (%)	Vit. B_9 (μg/g) C_{18} column	RSD (%)
Black flour	1.90	2.2	1.75	2.2
White flour	0.94	2.1	0.87	1.5
Rice	1.90	1.9	1.80	1.3
Semolina	1.00	1.5	1.10	1.9
Corn	0.02	3.0	0.02	2.4
Bread	0.89	2.5	1.00	1.2
Pasta	0.81	2.3	0.79	2.1
Gram	0.28	2.2	0.26	1.9
Borghol	0.60	1.9	0.57	1.3
Black lentil	1.20	1.1	1.30	1.1
Yellow lentil	0.39	3.1	0.42	1.4
Triticum durm	1.71	0.9	1.59	1.1
Triticum aestivum	1.20	1.2	1.4	0.8
Lima bean	2.20	2.1	2.20	2.0

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(*Received*: 18 March 2010; *Accepted*: 31 July 2010) AJC-8929