Determination of vitamins B_1, B_2 and B_9 in some vegetable samples:Cabbage , Beets ,Cauliflower and Lettuce By HPLC Method

HAMAD.M.ADRESS.HASAN[1], ABDULRRZIQ.S.A.MOHAMED[1] and Sajeda.A.Al-Gammie[2]
[1]Chemistry Department, Faculty of Science, Omar Al-Mukhtar University
[2]ChemistryDepartment, Faculty of Arts and Science (Derna), Omar Al-Mukhtar University

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Abstract:
The contents of B_1,B_2 and B_9 were estimated in some vegetable samples incliding: (Cabbage,Beets,Cauliflower and Lettuce) by HPLC technique.The concentrationswere measured from the calibration cueve also the validation methods were calculated. The evaluation of calibration model was based on the coefficient of determination (R^2).The linearity of the concentrations was studied according to values of slope and intercept of the regression line and the correlation coefficient (R^2).The fruitful linearity was obtained for the compounds between the concentrations and peak area , where the concentration which gave good linearity were ranged as following: 25 – 200 µg/ml ,5 – 25 µg/ml and 100 – 400 µg/ml for B_1 , B_2 and B_9 , respectively.
Keywords: Cabbage , Beets ,Cauliflower and Lettuce,Vitamins, HPLC

Introduction

Vitamins are small amount of organic molecules required for normal growth and maintenance of the human body. Except for pantothenic acid, which is a linear compound, most of them are aromatic substances. Plant products are usually a rich source of vitamins, but in animal organisms, they are formed only through food intake or the anabolic activities of microorganisms living in the intestine. Vitamins are usually concentrated in animal and plant tissues with higher metabolic activity. Therefore, the liver or kidneys are a more powerful source of vitamins than muscles, skin or other parts. Most of them perform their functions in the form of coenzymes or prosthetic groups of enzymes. Together with certain amino acids, vitamins constitute of the 24 organic compounds , which have been classified as dietary necessities. The vitamin plays a vital role in the metabolic process of the body (ie maintenance, growth, development and/or production of). But its lack or underuse can cause specific deficiency syndromes(Ozawa et al.,2001).

Foods extracted from plants and animals are the most important source of vitamins in human daily diet. However, vitamins are unevenly distributed among various food sources of plant and animal origin. Taking kinds of synthetic vitamins can eliminate vitamin deficiency. They are divided into two categories based on solubility. water-soluble vitamins are vit.B1, vit.B2, vit B3, vit B5, vit B6, vit B12, vitamins. C, folic acid and vitamins. H (Biotin) and fat-soluble vitamin vit. A, vit D, vit E. and vit K.

The B vitamins are named as thiamine (vitamin B1) riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6), pantothenic acid (vitamin B5), biotin (vitamin B7), folate (vitamin B9) and cyanocobalamin (vitamin B12).

These vitamins help to prevent art disease (Ozawa et al , 2001), brain disorder (Togay - Isikay et al ., 2001), mental illness (Linton et al.,2002; George and Chin, 1998) , autism (Lonsdale et al ., 2002) , to alleviate dysmenorrheal (Wilson and Murphy, 2001), protect against uremia (Hung et al ., 2001) , the renal disease (Frank et al ., 1999) , to

the diabetic neuropathy (Abbas et al .,1997),cures Beriberi (Twu et al ., 2003) and helps to the elderly population by improving sleep and increase energy (Wilkinson et al ., 1997) etc.

Metamizole (MET), Thiamine (B1) and Pyridoxin (B6) are active pharmaceutical ingredients frequently combined and widely used to relieve pain complaints caused by neuritis and neuralgia, especially on severe pain (Indonesian Pharmacist Association, 2011) Metamizole is pirazolon derivative having analgesic and antipiretic effects , it is commonly used to relieve acute pain (Zukowski and Kotfis, 2009).

Thiamine and pyridoxine are neurogenic vitamins that play an important role in the formation of energy metabolism required by brain cells. The combination of MET and vitamin B complex (ie thiamine and pyridoxine) will increase the potential synergy of analgesia-antipyretics (Rosales et al., 2005; Nikolova et al., 2012). Some analytical methods have been reported for the determination of MET, B1 and B6, whether used alone or in combination with other drugs. Various analytical methods for the determination of MET, such as electrochemistry and electrophoresis (Basaez et al., 2008), reflectance method (Weinert et al., 2007), spectrophotometry (Salih and Al-Sharook, 2008), HPLC (Altun, 2007), and liquid chromatography-mass spectrometry (LC/MS) were used for bioequivalence studies (Shep et al. , 2012).

This study was designed to develop a new analytical method on a mixture of water soluble vitamins :Thiamine (B_1), Riboflavin (B_2) and Folic acid (B_9) to simultaneous determination in some vegetable samples including (Cabbage , Beets, Cauliflower and Lettuce) by :RP – HPLC Method.

Material and Methods

This description of the methods and the apparatus which used in this study :

Apparatus Perkin Elmer HPLC Series PE-200 (USA) equipped with a P200 pump, solvent degasser DGU-3A, an automatic sampler AS200, Rheodyne injector with 200 µL loop, UV/VIS detector Series 200 with controlled wavelength at 262 nm and communication Network chromatography Interface Dot Link 600, a Brownlee BIO C18 reversed-phase analytical column, 5µm particle size, 250x4.6 mm dimension, Mettler Toledo balance 200.

Reagents and Solutions : All reagents used were of analytical-reagent grade. Thiamin (B_1) was purchased from (BDH) , Riboflavin (B_2) and Folic vitamins were purchased from (Sigma company).**They** were used **without** any further purification. Standard solution of folic acid was prepared by dissolving 100 mg of this vitamin in 1 ml sodium hydroxide solution (0.01 M), the solutions were shacked well then completed to 100 ml with distilled water.

This stock solution was kept in a refrigerator. On the other side the thiamin and riboflavin vitamins were prepared by dissolved 100 mg of each one in distilled water , the volumes were completed to 100 ml in measuring flasks .The supporting electrolyte used for both RP-HPLC was phosphate solution (0.04 mol.L^{-1} KH_2PO_4) adjusted to pH 7 with sodium hydroxide.

samples : The contents of vitamins were evaluated in the following vegetable samples (Cabbage , Beets , Cauliflower and Lettuce). All tested vegetable samples were purchased from local markets at El –Beda city.

Sample Preparations : The aqueous extraction was carried for the vegetable sample by taken 50 gram of each samples and transferd to beaker contenning 200 ml

distillig water then heated at (65 - 75 ^{0}C) until near drynees, then filtered through Whatman filter paper (# 41) and completed to 50 ml with distilled water. Further dilution is accomplished by transferring 1 ml of the previous solution to 25 ml measuring flask and completed to the mark with distilled water.

Procedure:

Thiamine (B_1):An aliquot of standard Thiamin solutions (2.5 - 25 µg/ml) were placed in a 10 ml measuring flask and completed to the mark with distilled water. The solution is then transferred to quartz cell with 10 mm path length the distilling water was used as blank.The unknown samples were measured directly without further dilution and the concentrations were calculated according to the calibration curve.

Riboflavin (B_2): An aliquot of standard Riboflavin (B_2) solutions (2.5 - 25 µg/m1) were placed in a 10 ml measuring flask and completed to the mark with distilled water. The solution is then transferred to quartz cell with 10 mm path length. The unknown **samples** were measured directly without further dilution and the concentrations were calculated according to the calibration curve.

Folic acid (B_9): An aliquot of standard Folic acid (B_9) solutions (20 - 150 µg/ml) were placed in a 10 ml measuring flask and completed to the mark with distilled water. The solution then transferred to quartz cell with 10 mm path length and the absorbance is recorded against water blank at wavelength 278 nm. The unknown samples were measured directly without further dilution and the concentrations were calculated according to the calibration curve .

Reverse Phase High Performance Liquid Chromatographic Analysis :

After series trials the mobile phase which selected in study was 0.04 mol l^{-1} KH_2PO_4 (pH =7) : acetonitrile, 75:25 .The flow-rate was 1 ml min^{-1}. The column was operated at room temperature (20 °C). The mobile was first degassed and (1 µl) Thiamin solution (20 -200 µg/ml) was injected into 200 µL loop and the column elute was monitored with a UV detector at 263 nm. The same conditions were applied on the other vitamins but by used concentrations of (5- 25 µg/ml and 100 – 500 µg/ml) for riboflavin and folic acid ,respectively. Identification of the studied vitamins in a sample was ascertained by comparing its retention time with that of standard solutions and their concentrations were calculated from the calibration curve of integrated peak areas versus the corresponding concentrations of standard solutions .

RESULTS AND DISCUSSION

The HPLC method:

Optimizing the conditions of separation:

After trials of investigations of different parameters including **p**H , mobile phase composition (various ratios of solvents) ,and flow rate ,the best retention time was chosen for separation of the studied vitamins . From the chromatogram shown in Figure (1), it is evident that the mobile phase consisting of (Buffer solution : acetonitrile) of ratio (75 : 25) gave good separation comparing with the other applied mobile phase ratios of (70 : 30) , (77 : 23) and (80 :20) , Figures (2 - 4). Also differ-

ent **p**H values were used during the separation including pH values of 4 , 6 and 7 (Figures 5 , 6 and 7).The obtained results showed that the pH value of 7 gave the good separation comparing with the other pH values of 4 and 6 , Figures (6) and (7) .

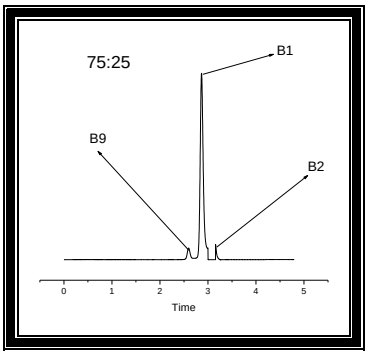

Figure (1): The effect of the mobile phase (75:25) on the separation**.**

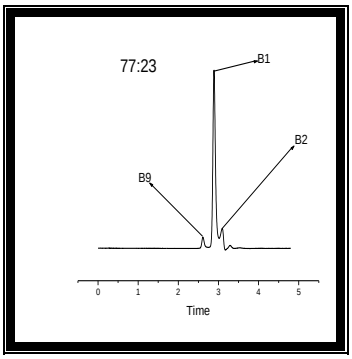

Figure (2): The effect of the mobile phase (77:23) on the separation.

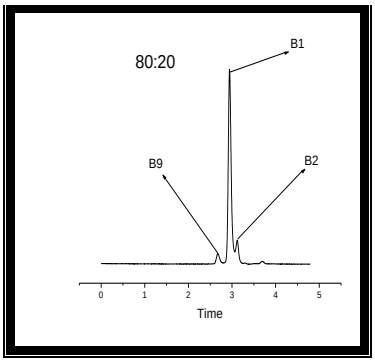

Figure (3): The effect of the mobile phase (80:20) on the separation.

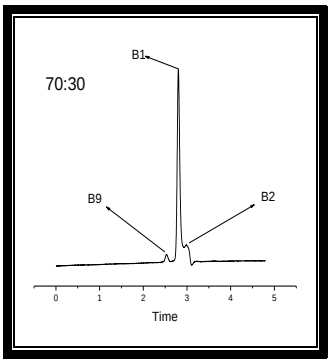

Figure (4): The effect of the mobile phase (70:30) on the separation.

Also the effect of pH values on the separation was studied by modified the applied pH values the results were shown in Figures (5 -7).

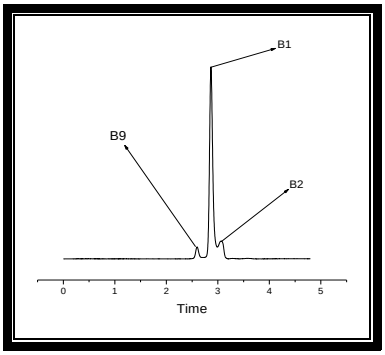

Figure (5): The effect of (PH = 4) on the separation of B_1, B_2 and B_9

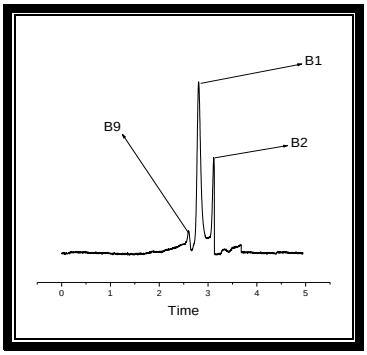

Figure (6): The effect of(PH= 6) on the separation of B_1, B_2 and B_9.

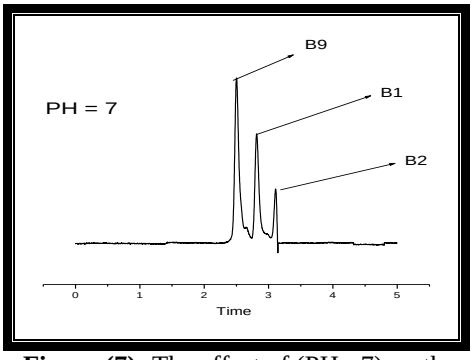

Figure (7): The effect of (PH= 7) on the separation of B_1, B_2 and B_9.

Also after series of attempts the total of chromatographic run time is 3.3 min , the flow rate is 1 min/ml which required to move the mobile phase from the injection loop to the column to the detector .On the other side the value of U.V which applied in the HPLC was chosen according to the λ_{max} value which obtained from the spectrophotometric measurements during this study,where the results showed that λ_{max} of the studied vitamins were : 263 , 264 and 278 nm for B_1 , B_2 and B_9 , respectively .The best optimizing conditions of separation which chosen in this study were shown in Table (1).

Table (1) : The best Optimizing conditions of separation.

Parameter	Value
PH	7
Mobile Phase	75 : 25
Wave length	262
Retention time (t_R)	3.3 min

The applied method was subjected to validation for various parameters including linearity , accuracy ,limit of detection(LOD), limit of quantification (LOQ) ,Suitability of method, precision and accuracy.

Linearity :

The linearity of the concentration against peak height was studied according to values of slope , intercept of the regression line and correlation coefficient (R^2) . The fruitful linearity was obtained for the compounds between the concentrations and peak area , where the concentration which gave good linearity were ranged as following : 25 – 200 µg/ml , 5 – 25 µg/ml and 100 – 400 µg/ml for B1 , B2 and B9 , respectively .Table (2) shows the linearity results of the studied vitamins

Table (2) : The linearity results of the HPLC method

Parameter Vitamin	Range	slope	intercept	R^2	Sy/x	LOD mg/ml	LOQ mg /ml
B1	25- 200	13539.16	18457.68	0.93	418375	0.092	0.309
B2	5 - 25	49200	-268000	0.96	147241	0.0089	0.029
B9	100 - 400	593.76	-85015	0.94	33423	0.168	0.562

The chromatograms and the calibrations curves of the studied vitamins were shown in the Figures of (8 - 13).

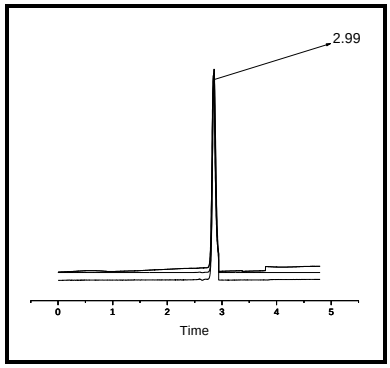

Figure (8): The chromatogram of standard solutions of Thiamine B_1 .

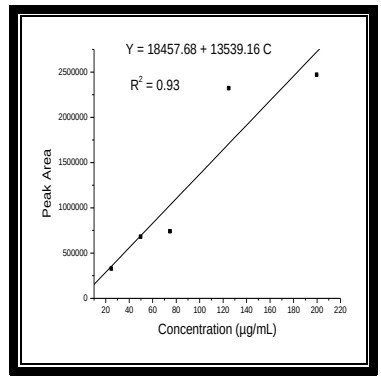

Figure (9): The standard curve of Thiamin (B1).

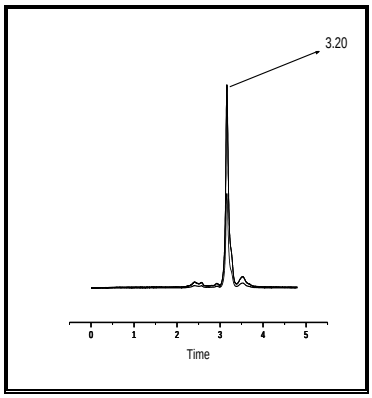

Figure (10): The chromatogram of standard solutions of Riboflavin

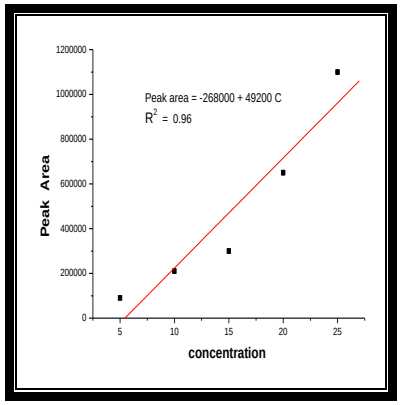

Figure (11): The standard curve of Riboflavin (B2)

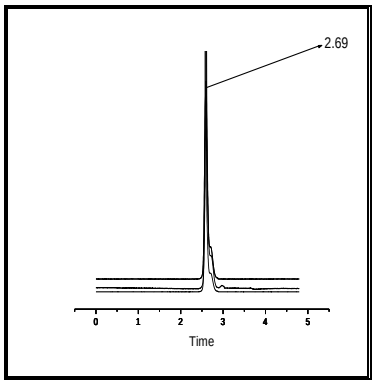

Figure (12): The chromatogram of standard solutions of Folic acid B9.

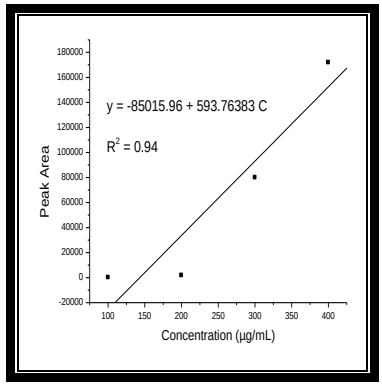

Figure (13): The standard curve of Folic acid (B_9).

Sensitivity of the method (LOD &LOQ) :

Sensitivity of the applied method was evaluated by calculation the values of limit of detection (LOD) and limit of quantification (LOQ). LOD, which is defined as the lowest active substance concentration that can be determined by a method, usually cannot be calculated precisely and accurately. On the other hand, LOQ is the concentration of the sample used in analysis that can be obtained with adequate precision and accuracy. These limits are estimated by the two following equations(Shep et al., 2012):

LOD = 3 Sy/x / S

LOQ = 10 Sy/x /S

where Sy/x is the residual standard deviation and S is the slope of the regression line. The LOD is calculated to be 0.092 , 0.0089 and 0.168 µg/ml . Whereas the LOQ values were 0.309 , 0.029 and 0.562 for B1 , B2 and B9 , respectively .

Applications :

The proposed HPLC method was applied to determine vitamins B1, B2 and B9 in some vegetable samples including: Cabbage, Beets, Cauliflower and Let-

tuce . The selected samples containing the studied vitamins alone or along with other vitamins. The applied method showed fruitful results ,where , complete separation was obtained . Three different vegetable samples were selected as application of the proposed methods ,including (Cabbage , Beets , Cauliflower and Lettuce) samples. The values of retention times of the vegetable samples were compared with the retention time of each vitamin of standard solutions . The results showed that the retention times of the vitamins were appeared at 2.67 ,3.0 and 3.2 min for B9 , B1 and B2 , respectively and agreed with the retention times of the standard solutions. The contents of the vitamins which obtained from HPLC method were illustrated in Table (3).

Table (3) : The contents of the studied vitamins in the selected samples

Parameter / Sample		Content of vitamins (ppm)
Beets	B1	80
	B2	59
	B9	245
Cauliflower	B1	57
	B2	33
	B9	120
Lettuce	B1	43
	B2	90
	B9	168
Cabbage	B1	130
	B2	180
	B9	200

The three water-soluble vitamins (Thiamine , riboflavin and folic acid) were separated and analyzed by RP - HPLC. The method provided a rapid , accurate and reliable method .

The developed method was further applied to pharmaceutical or other samples . The binary eluent system used for water-soluble and the isocratic eluent system used for soluble vitamins provide good separation high selectivity and resolution within a minimum analysis time of 3.3 min . The simplicity of the procedure should make it highly desirable for quality control of multi-vitamin products in food industries , plant extractions and drug intergradients

The advantages of this method are relate into different factors :

1.It is a new method for the mobile phase ratio.

2.Rapid , where the separation time is completed within 3 min comparing with many of published methods

3.Sensetive to determine different types of soluble water vitamins , especially which selected in this study (Some of B vitamins).

Conclusion

The use of HPLC for the simultaneous analysis of B1, B2 and B9 can be an alternative technique for quality control of the Pharmaceutical formulations.The present HPLC method for the determination of B1, B2 and B9 was successful for a wide range of concentration and was cheap, easy and rapid. As the standard deviation values obtained from the analysis were satisfactory, it can be concluded that the method is sufficiently sensitive and reproducible in the routine analysis of vitamin B1, B2 and B9.

results obtained with HPLC ,indicate that the method is simple and is not time consuming.

REFERENCES

1-Abbas, Z and Swai, A.(1997) Thiamine in the Treatment of Diabetic Neuropathy. East African Medical Journal 1997., 12 :8-803.

2-Altun, M.(2002) HPLC method for the analysis of paracetamol, caffeine and dipyrone. Turk J Chem., 21(5) : 8-26.

3- Basaez, L; Peric, I; Jara, P and Soto, C. (2008) Electrochemical and electrophoretic study of sodium metamizole. J Chil Chem.,53(3) : 5-1572.

4- Frank, T; Bitsch, R; Maiwald, J; and Stein, G.(1999) Thiamine and Renal Disease. International Journal of Clinical Pharmacology & Therapeutics., 37(9) :449-55.

5-George, S and Chin, C. (1998) Thiamine Supplements and Alcoholism. Medical Journal of Malaysia.,(3) : 53 -223 .

6-Hung, S; Hung, S; Tarng, D; Yang, W; Chen, T and Huang, T.(2001) Thiamine Deficiency and Brain Swelling. American Journal of Kidney Diseases., 38(5) : 941-7.

7- Indonesian Pharmacist Association. InformasiSpesialite Obat Indonesia. Jakarta: PT ISFI Penerbitan; 2011.

8-Linton, C; Reynolds, M and Warner, N.(2002) Using Thiamine to reduce post-ECT Confusion. International Journal of Geriatric Psychiatry., 17: 189-92.

9-Lonsdale, D; Shamberger ,R and Audhya, T .(2002) Treatment ofAutism with Thiamine. Neuroendocrinology Letters,(4)23 : 8-303 .

10- Nikolova, I;Tencheva, J; Voinikov, J;Petkova, V; Benbasat, N and Danchev, N. (2012) Metamizole: A ReviewProfile of a Well-Known "Forgotten" Drug. Part I: Pharmaceutical and Nonclinical Profile. BiotechnolBiotechnol Equip , (6):3329-37.

11- Ozawa, H; Homma Y; Arisawa, H; Fukuuchi, F; and Handa S .(2001) Heart Failure due to Thiamine Deficiency. Nutrition, 17(4) : 2 -351 .

12- Rosales, F; Santillan, R and Reyes-Garcia, G.(2005) interaction between acetaminophen or metamizol and B vitamins in the formalin test. Drug Dev Res,66(4) : 9 -286

13- Salih, E and Al-Sharook, M. (2008) Spectrophotometric assay of dipyrone in pharmaceutical preparations viaoxidative coupling reaction with m-toluidine and potassium hexacyanoferrate (III). J Edu Sci ., 21 (2) : 36-45.

14- Shep, D; Ojha, R; Rathod, R and Patel, S. (2012) Bioequivalence study of two oral formulations of metamizole 500 mg in healthy volunteers. Int J Pharm Sci Res., 3(6): 1749-52.

15- Togay – Isikay, C. (2001) Yigit A.,Mutluer N. Thiamine: Disorder. Australian & New Zealand Journal of Obstetrics &Gynaecology., 41(4):453-6.

16- Twu, S; Chiou, S; Pan, W; Chang, H and Serdula, M (2003). Vitamin B1 Cures Beriberi. Public Health Reports, 118(1):59-64.

17- Weinert, P; Pezza, L and Pezza H . (2007) A Simplified Reflectometric Method for the Rapid Determination of Dipyrone in Pharmaceutical Formulations. J Braz Chem Soc, 18(4):846-54.

18- Wilkinson, T; Hanger, H; Elmslie, J and George, P.(1997) Sainsbury R.Thiamine Deficiency in the Elderly American Journal of Clinical Nutrition ., 4 : 8-925.

19- Wilson, M and Murphy, P.(2001) Vitamin B1 Helps Reduce Menstrual Cramps. Cochrane Database of Systematic Reviews:CD002124.

20- Zukowski, M and Kotfis, K.(2009) Safety of metamizole and paracetamol for acute pain treatment. Anestezjo Intens Ter,41(3):170-5 .

تقدير الفيتامينات B1 و B2 و B9 في بعض عينات الخضار: الكرنب، البنجر، القرنبيط، الخس بطريقة كروماتوجرافيا السائل ذات الكفاءة العالية

حمد محمد ادريس حسن [1] وعبدالرزيق س ا محمد [1] وسجدة أ الجمعي [2]
1 قسم الكيمياء بكلية العلوم جامعة عمر المختار
2 قسم الكيمياء بكلية الآداب والعلوم (درنة) جامعة عمر المختار
abdelraziq.soliman@omu.edu.ly

الملخص

تم تقدير محتويات B1 وB2 وB9 في بعض العينات النباتية (الكرنب، البنجر، القرنبيط، الخس) بتقنية الكروماتوجرفيا السائلو ذات الكفاءة العالية (HPLC). تم قياس التركيز من منحني المعايرة أيضًا تم حساب طرق التحقق من الصحة. استند تقييم نموذج المعايرة على معامل التحديد (R^2)، ودُرِسَت خطية التراكيز على أساس قيم انحدار وتقاطع خط الانحدار ومعامل الارتباط (R^2)، تم الحصول على الخطية المثمرة للمركبات. بين التركيزات والمسحة تحت السن حيث تراوح التركيز الذي أعطى خطية جيدة كالتالي: 25-200 ميكروغرام/مل، 5-25 ميكروغرام / مل و100-400 ميكروغرام / مل لـ B1 وB2 وB9 على التوالي.

كلمات مفتاحية: ملفوف، شمندر، قرنبيط، خس، فيتامينات، HPLC