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Determination of water soluble vitamins in Egyptian Honey by RP-HPLC

Ahmad M. Farag[1*], Mahmoud S. Rizk[1], Hamdy A. El-Bassel[2], Mona H. Youssif[1**] and Fatehy M. Abdel-Haleem[1]**
1- Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt.
afarag49@yahoo.com, ms_rizk@yahoo.com, fatehy@sci.cu.edu.eg
2-Department of Nutritional Biochemistry and Metabolism, National Nutrition Institute, Cairo, Egypt. h.elbassel17@gmail.com,
**mona.hanafy83@gmail.com

ABSTRACT

The purpose of this study is to evaluate water soluble vitamins (C, B_1, B_2, B_3, B_6, B_9 and B_{12}) in honey from honey bees feed on Spring flowers, Sweet marjoram, Sun flower, Clover flowers, Citrus fruits and Black seed using gradient RP-HPLC methods with PDA detector. The highest levels of vitamins C, B_1, B_2, and B_6 were observed in citrus fruits honey, sunflower honey, black seed honey and spring flower honey, respectively. The highest levels in vitamins B_3, B_9 and B_{12} were observed in sweet marjoram honey, where the lowest levels for these vitamins were observed in clover flower honey. The lowest levels for vitamins B_2 and B_9 were observed in black seed honey. The lowest levels for vitamins C and B_1 were observed in spring flower honey and citrus fruits, respectively. These results indicated the presence of a significant difference in water soluble vitamins content depending on the type of Egyptian honey.

Key words: HPLC, PDA, Water soluble vitamins, Egyptian honey.

INTRODUCTION

Vitamins are organic nutrients required by the body to ensure normal growth and metabolism (Semba, 2012). Vitamins are classified as micronutrients which exist in food in small quantities, and macronutrients as protein, carbohydrates and fats (Rucker *et al.*, 2007). Although vitamins are essential for the prevention of a number of diseases and the maintenance of good health, the human body cannot make vitamins and it must get them from different foods which are considered as vitamin supply (Semba, 2012; Rucker *et al.*, 2004, 2007). The importance of vitamins in nutrition was understood in the 1920s and 1930s, where lack of them can cause various diseases in humans, and small concentrations are required to maintain good health (Semba, 2012; Rucker *et al.*, 2004, 2007). Because of the critical role of vitamins in nutrition, qualitative and quantitative analyses are important issues and a challenging task for food manufacturers.

Water soluble vitamins are thiamine (B_1), riboflavin (B_2), pyridoxine HCL (B_6), cyanocobalamin (B_{12}), nicotinic acid (B_3), folic acid (B_9), pantothenic acid (B_5), and vitamin C. Complex vitamins B and vitamin C cannot be stored in human tissues. Their excess is excreted with urine but excess amounts of fat soluble vitamins stored in adipose tissues and in the liver (Rucker *et al.*, 2004).

Honey is a sweet food made by bees from the flowers nectar or honeydew droplets (Ajibola *et al.*, 2012). It has been found to be beneficial to people suffering from anemia (Ajibola *et al.*, 2007) where it improves hemoglobin concentration and increases erythrocyte count and elevated hematocrit in the honey eaters. Also, it was reported that honey enhanced hematology and immune response in rats fed with 10% honeydew honey supplemented diet (Chepulis and Compl, 2007). Subjects

administered with two honey treatments in a Californian study show that honey eaters have the benefit of haematoprotection in addition to blood proliferation (Schramm *et al.*, 2003). The researchers observed that the aqueous portion of the blood (plasma) is protected by honey. This agrees with the fact that most of the antioxidant components in processed honey are water soluble.

In summarizing the facts that honey can be considered a satisfactory immuno-nutrient, some workers opine that the oral administration of natural honey can stimulate and increase the production of antibody during primary and secondary immune responses against the T-cells of the thymus-dependent as well as the thymus independent antigens (Al-Waili and Haq, 2004). Honey essentially consists of a supersaturated solution mostly from fructose and glucose; it contains about 90% carbohydrates and minor substances as polyphenols, enzymes, organic acids, flavonoids, minerals, amino acids and proteins, but the water-soluble vitamins are the main products (Abdel-Haleem *et al*., 2016 ; Rizk and Abdel-Haleem, 2010). Different analytical methods could be used for the determination of the different species, including drugs and vitamins (Abdel-Haleem *et al*., 2016; Rizk and Abdel-Haleem, 2010). Electrochemical methods are of the best methods, as it is simple, rapid for routine analysis, cost-effective, applied in a wide concentration range and of high sensitivity (Abdel-Haleem *et al*., 2016; Rizk *et al.*, 2017).

However, in case of real samples that may include vitamins and proteins, adsorption to the electrode surface is a serious problem (Buhlmann *et al*., 1997). On the other hand, the common official analytical method is non-specific, tedious and time consuming as these methods involve pretreatment of the sample through physical, biological and complex chemical reactions to eliminate the interferences found (Indyk *et al*., 2002). it followed by individual methods for each different vitamin. The determinations of vitamins B and vitamin C in food were performed by spectrophotometry (Revanasiddappa and Veena, 2008), electrochemical method (Marszall *et al*., 2005; Mimica *et al*., 2002), capillary electrophoresis (Fotsing *et al.*, 1997) and HPLC for more complicated mixtures of two or more vitamins (Ramla, 2016; Rokayya *et al*., 2014; Ciulu *et al*., 2011; Ekinci & Kadakal, 2005) with gradient elution program. The separations were occurred on normal-phase, ion-pairing and reversed-phase chromatography columns.

In the present work, a simultaneous determination of B complex vitamins (B_1, B_3, B_6 B_9, B_2 and B_{12}) and vitamin C in honey was proposed using a simple gradient program by setting the wavelength at 272 nm. Also, the chromatographic system developed clearly separates the seven analytes from their degradation products.

MATERIALS AND METHODS

1. Apparatus and chemicals

HPLC (Shimadzu LC-20, Japan), Centrifuge (Pro-Research, UK), pH-meter (Jenway3310, British), Mixer (Falk, Germany) and Ultra-sonic bath (Elma, Germany) were used in this study.

Honey samples were collected from the Faculty of Agriculture, Cairo University. Samples were collected in glass bottles and stored in dark at 25 °C. Honey samples were spring flowers, sweet marjoram, Sun flower, clover flowers, citrus fruits and Black seed.

All chemicals were of the highest purity available analytical grade and all solvents in this study were HPLC-grade and obtained from sigma Aldrich (Germany).

2. Preparation of vitamins solution

The mixtures of water soluble vitamins were prepared by dissolving 200 mg of Thiamine hydrochloride, nicotinic acid, folic acid, pyridoxine hydrochloride,

vitamin C, cyanocobalamin, riboflavin in 1L of 4% trichloro acetic acid (TCA). The standard solution was kept in the dark at 4 °C and was prepared fresh daily. This stock was used for preparation of more dilute solutions by appropriate dilutions.

a. Extraction of samples

Five grams of homogenized honey were weighed, completed to the mark in 10 ml measured flask by using 4% TCA, mixed for 5 min, centrifugated for 15 min (6000 RPM), filtered by using 0.45 µm filter membrane and injected in HPLC instrument.

b- Method

High performance liquid chromatographic method was used for the determination of water-soluble vitamins. Separation was performed on a Waters spherisorb ODS2 (250 mm × 4.6mm, 5µm) column. Gradient elution was performed with a mobile phase consisting of 0.2 g heptane-1-sulfonic acid sodium salt in deionized water: methanol (90:10, v/v) of pH 3 (solvent A), and 0.2g heptane-1-sulfonic acid sodium salt in deionized water: methanol (30:70, v/v) of pH 3 (solvent B) at the flow rate 0.6 ml min^{-1}. Starting with 100 % solvent A then the composition was changed gradually during next 30 min to reach 100% of solvent B. The measurements were carried out at wavelength 272 nm, at room temperature with 0.6 ml min^{-1} flow rate.

RESULTS AND DISCUSSION

Honey consists of a mixture of complex compounds (flavonoids, phenolic acids and amino acid); so, separation methods as HPLC with UV-VIS detectors or PDA detector, are the most suitable for these complex compounds.

Selection of the appropriate column

There were many different types of chromatographic columns that have been used for separation of some water-soluble vitamins; intersil ODS (250 mm × 4.6 mm × 5 µm) and a waters spherisorb ODS2 (250 mm × 4.6 mm × 5 µm) chromatographic columns were tested (Figs. 1A & B). The present study showed that Waters spherisorb ODS2 (250 mm × 4.6 mm × 5 µm) was good for separation of the seven analytes in the chromatogram (Fig.1B).

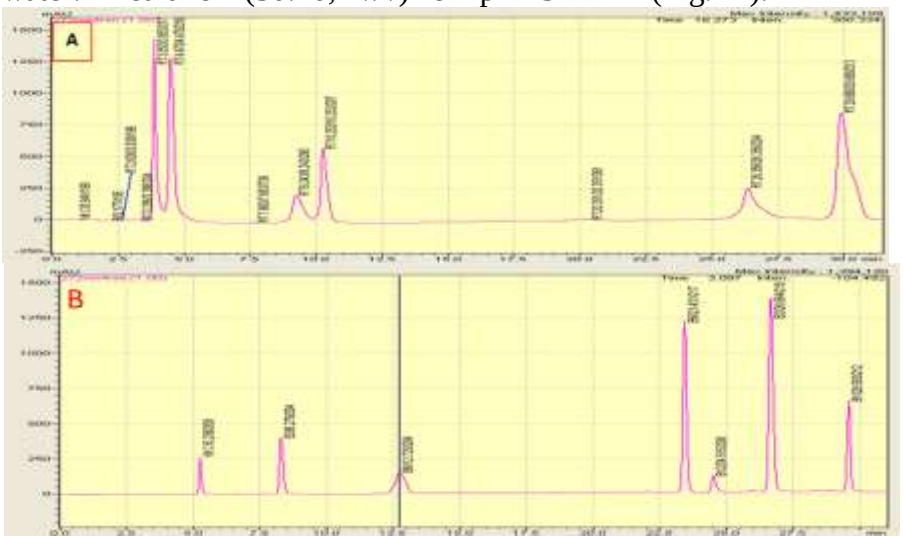

Fig. 1: Chromatogram for vitamin c and different vitamins B using intersil ODS column (A), and water spherisorb ODS2 column (B).

Resolution measurement of waters spherisorb ODS2 column

The results listed in Table (1) show the column parameters for an optimum separation of water soluble vitamins. The capacity factor [$\mathbf{K= (RT_1-RT_0) / RT_0}$], separation factor $\mathbf{S=k_2/k_1}$ and the resolution [$\mathbf{R=2(RT_2-RT_1)/ (W_2+W_1)}$] were ranged in (1-9.9), (1.1-2) and (1.5-7), respectively, for water soluble vitamins separated on Waters spherisorb ODS2 column.

Table 1: Column parameters for an optimum separation of water soluble vitamins.

Compound	RT min	k	S	W	N	R
Vit C	5.1	1.0	----	0.6	1156	----
Vit B_3	8.0	2.0	2.0	0.6	2844	4.8
Vit B_6	12.2	3.7	1.8	2.0	595	3.2
Vit B_9	22.0	7.46	2.0	0.8	12100	7.0
Vit B_{12}	24.5	8.4	1.1	1.0	7744	2.7
Vit B_2	26.0	9.0	1.1	1.0	10816	1.5
Vit B_1	28.5	9.9	1.1	1.0	12996	2.5

Retention times (RT), capacity factors (K), separation factors(S), Column efficiency (N), Resolution (R) and peak width (W) show optimum condition for separation of water soluble vitamins.

The column selectivity, originally called the separation factor (S), is defined as the ratio of the capacity factors of two adjacent peaks. When the value of $S \geq 1$, this means that we obtained base lines separation for all the eluted mixtures (Lioyd *et al.*, 2011). A resolution value of 1.5 or greater between two peaks will ensure that the sample components are well separated (Lioyd *et al.*, 2011). The separation mechanism in Waters spherisorb ODS2 column depends on the hydrophobic binding interaction between immobilized hydrophobic ligand of the stationary phase and the R groups attached to the organic compound. The different values of retention times due to interaction between different R groups of organic compounds and two octadecyl groups of the stationary phase governed the mechanism of the retention time (Lioyd *et al.*, 2011).

Optimization for separation of some water soluble vitamins.

The optimization work mainly depends on the choice of UV wavelength and the addressed program of the chromatographic elution, to maximize both resolution and sensitivity. So, the contents of mobile phases were 0.2 g heptane-1-sulfonic acid sodium salt in deionized water: methanol (90:10, v/v) adjusted to pH 3 (solvent A), and 0.2g heptane-1-sulfonic acid sodium salt in deionized water: methanol (30:70, v/v) adjusted to pH 3 (solvent B).

Table 2: Three different gradient profiles for simultaneous vitamins separation by RP-HPLC.

Time / Solvent	Gradient-1(A)				Gradient-2(B)			Gradient-3(C)		
	0	15	30	50	0	15	37	0	15	30
solvent A%	90	40	10	10	92	47	3	100	50	0
Solvent B %	10	60	90	90	8	53	97	0	50	100

It was obvious from Table (2) and Figure (2) that gradient-3 was preferred because a resolution value of 1.5 or greater between two adjacent peaks will ensure that the sample components are well separated to a degree at which the area of each peak may be accurately measured and give vitamin C and six vitamins B with good resolution.

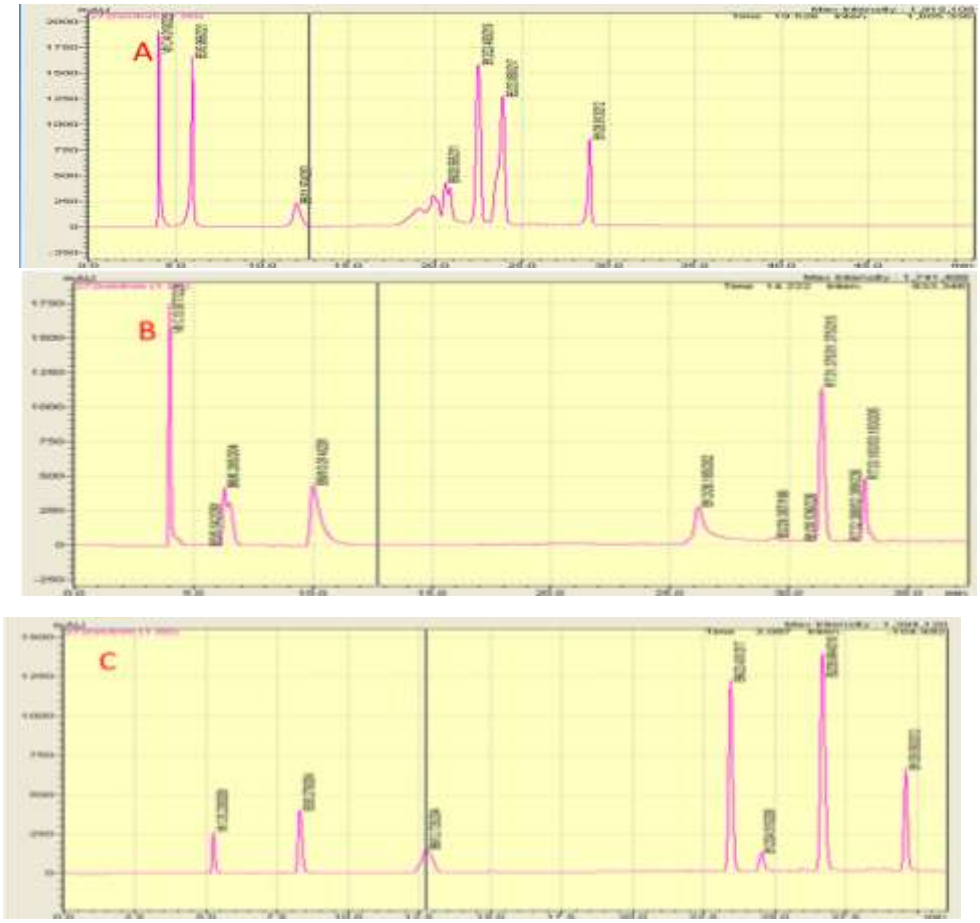

Fig. 2: **Chromatograms for RP-HPLC simultaneous vitamins separation gradiant-1(A), gradiant-2 (B) and gradiant-3 (C)**

The effect of pH on the retention time of some water-soluble vitamins standards

In the present study the effect of different pH values on the retention time of standards was studied by using two solvents (A and B); the pH of these solvents varied between ranges 2-7 by adding a few drops of sodium hydroxide or acetic acid. It was not possible to cover pH range less than 2 and more than 8 due to instability of the packing over the region since alkaline solution dissolves the silica support and at low pH breaks the Si-O linkage.

The curves show change of retention time of some water-soluble vitamins with the change of pH of mobile phase. The decrease in the retention time with pH increase is in accordance with the faint acidic pH of the honey (3-6), which maintains the honey acidic groups in the non-dissociated neutral form that in turn facilitates the interaction with the column of the low polarity and increase the retention time (Molnar, 1996). The optimum pH obtained for best separation of water soluble vitamins is at pH 3, because a resolution value of 1.5 or greater between two adjacent peaks will ensure that the sample components are well separated to a degree at which the area of each peak may be accurately measured (Fig.3).

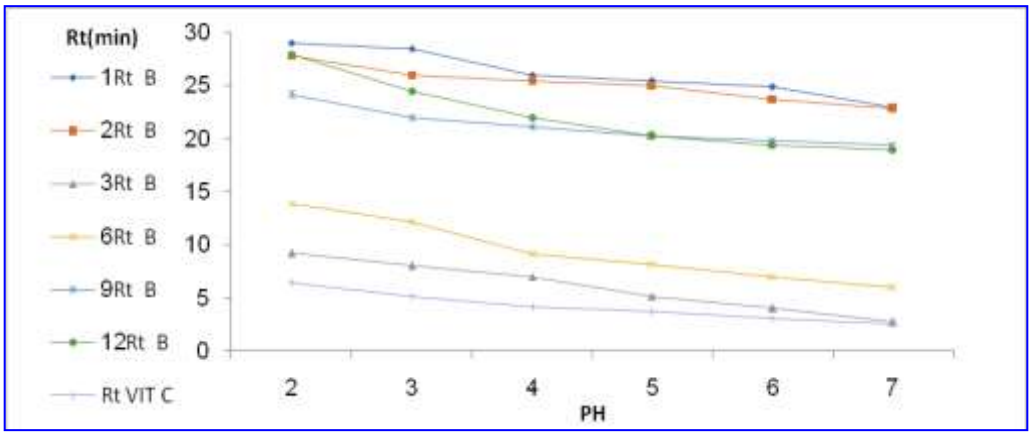

Fig 3: Effect of pH on retention time (RT) of soluble vitamins; B_1, B_2, B_{12}, B_9, B_6, B_3 and vit. C.

The effect of flow rate on separation of water soluble vitamins

HPLC column is affected with change of flow rate because of changes of pressure and analysis time (Ciulu *et al.*, **2011)**. A high flow rate may reduce the analysis time but may adversely affect the quality of chromatography, as it may not permit sufficient time for analyte separation and interact with stationary phase. Table (4) and Figure (4) show that, the retention time of water soluble vitamins changes with the flow rate (0.6 ml/min to 1.2 ml/min); acceptable flow rate is (0.6 ml/min) which gave resolution value of 1.5 or greater between two adjacent peaks and this indicated that the sample components are well separated, but high flow rate not sufficient for separation of folic acid and cyanocoblamin, thiamin and riboflavin, and ascorbic acid and nicotinic acid, and give resolution value less than 1 between two adjacent peaks.

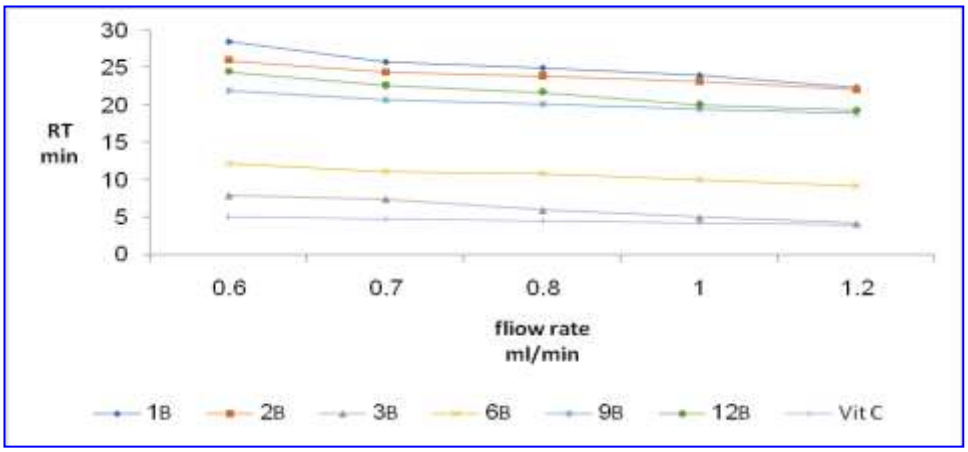

Fig 4: retention time as a function of flow rate for some water soluble vitamins; B_1, B_2, B_{12}, B_9, B_6, B_3 and) vit. C.

Calibration curves and method validation

The calibration curves were plotted for the different vitamins (Fig. 5) and showed excellent R values for the different vitamins, which indicates the applicability of the proposed method with high sensitivity.

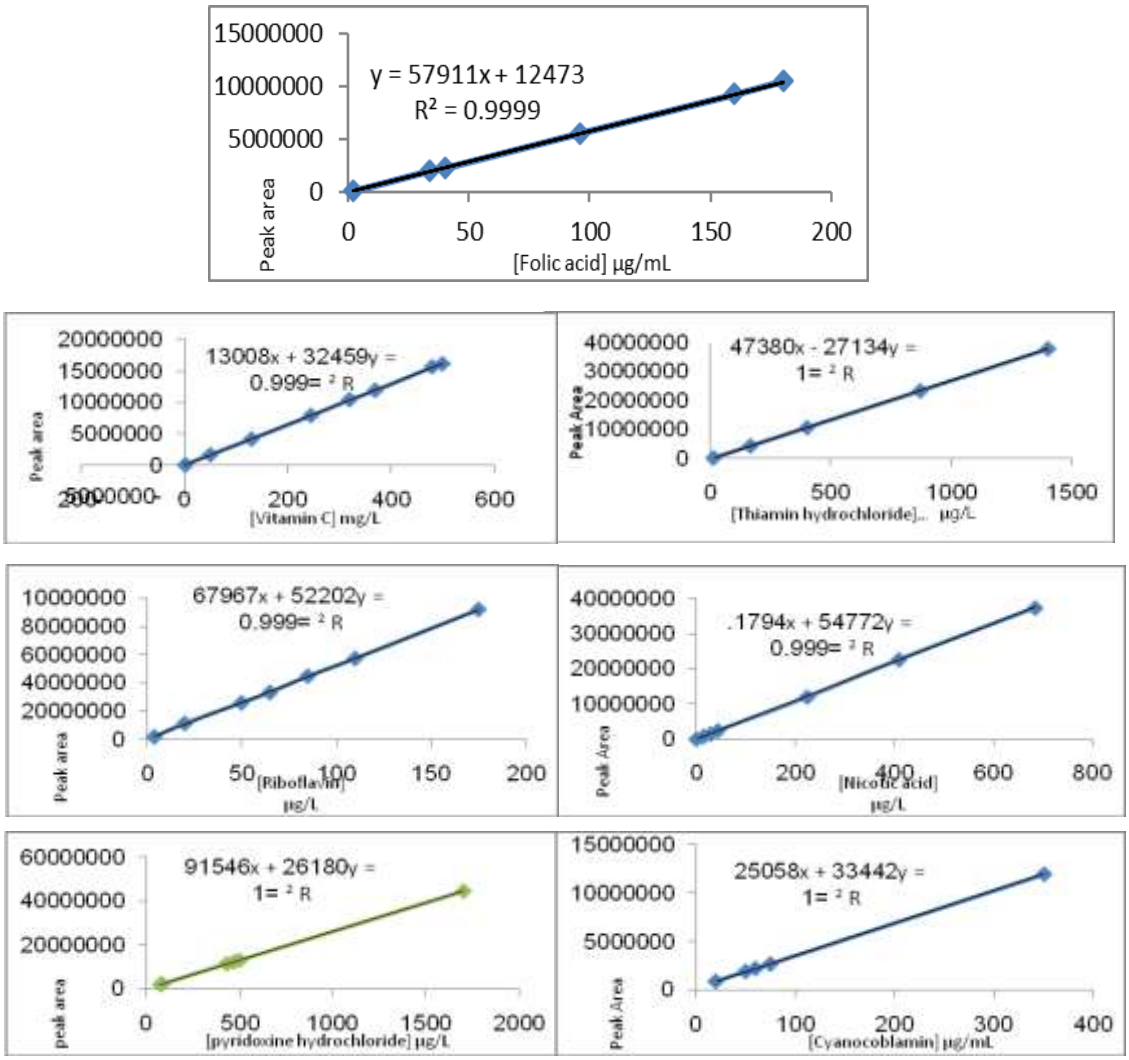

Fig. (5): calibration curve for water soluble vitamins.

Also, method validation studies were performed by measuring basic parameters such as precision, accuracy, linear region, limits of detection (LOD), and quantification (LOQ) ICH Harmonized, and recovery (Tables 3 & 4).

Table (3): Method of validation for determination of different vitamins with the proposed method.

Vitamin	Rt (min)	LOD	LOQ	Accuracy	Linear range
B_1(µg/L)	28.5	4.795	14.532	101.62±3.60	12-1400
B_2(µg/L)	26.0	2.412	7.3084	99.64±5.04	4-175
B_3(µg/L)	8.0	5.117	15.505	99.78±2.18	1.6-685
B_6(µg/L)	12.2	4.921	14.912	100.23±0.64	75-3150
B_9(µg/L)	22.0	1.064	3.225	98.04±5.66	2-540
B_{12}(µg/L)	24.5	8.380	25.395	100.30±1.98	20-350
Vit. C (mg/L)	5.1	3.230	10.768	100.22±2.14	1.5-500

Detection limits of vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) were 4.795, 2.412, 5.117, 4.921, 1.06, 8.380 µg/L and 3.230 mg/L, respectively, where the quantification limits were 14.532, 7.308, 15.505, 14.912, 3.225, 25.395 µg/L and 10.768 mg/L, respectively, with recovery% in the range of 98-102%.

Table (4): Concentration of Water soluble vitamins compounds in honey bee under study.

vitamins / honey	B_1 mg/kg	B_2 mg/kg	B_3 mg/kg	B_6 mg/kg	B_9 mg/kg	B_{12} µg/100g	C mg/kg
Spring flowers	--------	2.3±0.4	----------	3.73±0.9	1.98±0.9	0.83±0.07	0.6 ±1.31
Sweet marjoram	2.9±0.65	3.5±1.02	2.83±0.9	3.01±0.5	2.39±3.6	4.6±1.66	1.4±3.5
Sun flower	4.3±0.81	1.5±0.55	2.5±0.55	1.9±0.45	1.0 ±2.87	1.026±.43	----------
Clover flowers	1.18±0.59	--------	1.8±0.41	1.73±0.6	0.99±1.5	0.6±0.02	2.8±2.9
Citrus fruits	0.98±0.47	1.66±0.22	2.86±1.0	2.9±0.95	1.5±2.7	1.8±0.61	3.7±4.02
Black seed	2.1±0.74	1.06±0.35	4.1±1.44	1.9±0.64	0.74±1.8	---------	----------

Seven water soluble vitamins were measured in different types of honey sample which showed various concentrations (Table 4). The highest level for vitamin C in citrus fruits honey was 3.75 mg/kg, the highest level for vitamin B_1 in sunflower honey was 4.3 mg/kg, the highest level for vitamin B_2 in sweet marjoram honey was 3.5 mg/kg, the highest level for vitamin B_3 in black seed honey was 4.1 mg/kg, the highest level for vitamin B_6 in spring flower honey was 3.73 mg/kg, the highest level for vitamin B_9 in sweet majoram honey was 2.39 mg/Kg and the highest level for vitamin B_{12} in sweet majoram honey was 4.6 µg/100gm. While the lowest levels for vitamin C in spring flowers honey was 0.6 mg/kg, for vitamin B_1 in citrus fruits was 0.98 mg/kg, for vitamin B_2 in black seed honey was 1.06 mg/kg, for vitamin B_3 in clover flowers honey was 1.8 mg/kg, for vitamin B_6 in clover flower honey was 1.73 mg/kg, for vitamin B_9 in black seed honey was 0.74 mg/kg and for vitamin B_{12} in clover flowers honey was 0.6 µg/100g. In a study of (19) the highest level in honey samples for vitamin C was 5.8 mg/Kg in eucalyptus honey, B_2 was 9.2 mg/Kg in thistle honey, B_3 was 27.8 mg/kg in thistle honey and B_6 was 7 mg/kg in eucalyptus honey.

Conclusion

For quick, simultaneous and simple determination of seven water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) in Egyptian honey, gradient RP-HPLC method was developed and validated in terms of linearity, sensitivity and accuracy. Detection limits were 4.795, 2.412, 5.117, 4.921, 1.06, 8.380 µg/L and 3.230 mg/L,

respectively, and quantification limits of 14.532, 7.308, 15.505, 14.912, 3.225, 25.395 µg/L and 10.768, for vitamins B_1, B_2, B_3, B_6, B_9, B_{12} and C, respectively, with good linearity and good correlation coefficients (R^2). Mean recoveries obtained were in the range of 98-102% which ensures the success of the method. The extraction and separation using high-performance liquid chromatography (HPLC) exhibited high response, sensitivity and fast separation. The overall concentration of the seven analytes never increased 40 mg /kg and often appeared to be significantly dependent on the type of honey. According to these recommendations, the Sweet marjoram honey is good source of vitamins B_2, B_6 and B_{12}, and sun flower, Black seed, spring flowers and citrus honey are good sources of vitamin B1, B3, B6 and vitamin C, respectively.

REFERENCES

Abdel-Haleem, F.M.; Badr, I. and Rizk, M.S. (2016). Potentiometric anion selectivity and analytical applications of polymer membrane electrodes based on novel Mn (III)and Mn (IV) Salophen Complexes. Electroanal., 28 (12): 2922.

Abdel-Haleem, F.M.; Saad, M. and Rizk, M.S. (2016). Development of new potentiometric sensors for the determination of proguanil hydrochloride in serum. J. Chin. Chem. Lett, 27(6): 857

Ajibola, A.; Idowu, G.; Amballi, A.; Oyefuga, O. and Iquot, I. (2007). Honey and its Anti-Inflammatory, Anti-Bacterial and Anti-Oxidant Properties, J. Biol. Sci. Res.; 2: 67

Ajibola, A.; Chamunorwa, J.P. and Erlwanger, K.H. (2012). The nutritional profiles, including its use in infant and children feeding reported in different literatures. Nutrition & Metabolism 9: 61. DOI: 10.1186/1743-7075-9-61

Al-Waili, N. and Haq, A. (2004). Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune response, J. Med. Food, 7(4): 491.

Buhlmann, P.; Pretsch, E. and Bakker, E. (1997). Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 1. General Characteristics. Chem. Rev., 97(8): 3083-3132.

Chepulis, L. and Compl, J. (2007). The Effects of Honey Compared With Sucrose and a Sugar-free Diet on Neutrophil Phagocytosis and Lymphocyte Numbers after Long-term Feeding in Rats. J. Med., 4 (1): 1.

Ciulu, M.; Solinas, S.; Floris, I., Panzanelli, A.; Pilo, M.I.; Piu, P.C.; Spano, N. and Sanna, G. (2011 RP-HPLC determination of water-soluble vitamins in honey, Talanta, 83: 924.

Ekinci, R. and Kadakal, C. (2005). Determination of seven water-soluble vitamins in tarhana, a traditional Turkish cereal food, by high - performance liquid chromatography. Acta Chrom., 15(15): 289.

Fotsing, L.; Fillet, M. and Bechet, I. (1997). Determination of six water-soluble vitamins in a pharmaceutical formulation by capillary electrophoresis. J. Pharm. Biomed. Anal, 15: 1113.

ICH Harmonized (2005). Tripartite Guide Line Validation analytical procedures: text and methodology Q2 (R1), Parent Guidelines on Methodology.

Indyk, H.; Persson, B.; Caselunghe, M.; Filouzi, E. and Wollard, D. (2002). Determination of vitamin B12 in milk products and selected foods by optical biosensor protein-binding assay: method comparison, J. AOAC Intl., 85: 72.

Lioyd, R.; Joseph, J. and John, W. (2011). Introduction to Modern Liquid Chromatography, 3rd ed. (Wiley).

Marszall, M.L.; Lebiedzinska, A.; Czarnowski, W. and Szefer, P. (2005). High-performance liquid chromatography method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection. J. Chrom. A, 1094 (1-2): 91

Mimica, D.; Bedioui, F. and Zagal, J.H. (2002). Reversibility of the L-cysteine/L-cystine redox process at physiological pH on graphite electrodes modified with coenzyme B12 and vitamin B12. Electrochim. Acta, 48: 323

Molnar, I. (1996). Validation of robust chromatography methods using computer - assisted method development for quality control, LC-GC. Int., 9: 800.

Ramla, A. (2016). Determination of Water Soluble Vitamins [(B group: B1, B2, and B6) by RP- HPLC. J. Chem. and Materials Res., 8(10): 8 Revanasiddappa, H. and Veena, M. (2008). Sensitive spectrophoto-metric method for the determination of ascorbic acid E. J. Chem., 5: 10-15

Rizk, M.S. and Abdel-Haleem, F.M. (2010). Plastic membrane electrodes for the determination of flavoxate hydrochloride and cyclopentolate hydrochloride. J. Electrochim. Acta, 55 (20): 5592

Rizk, M.S.; Badr, I. and Abdel-Haleem, F.M. (2017). Potentiometric Determination of Ciprofloxacin in Physiological Fluids Using Carbon Paste and Nano Composite Carbon Paste Electrodes. Electroanal., 29 (4): 1172.

Rokayya, S.; Yang, L.; Baokun, Q., Hengnan, W.; Qiaozhi, Z.; Feifei, H.; Ying, M.; Jing, J. and Lianzhou, J. (2014). HPLC Analysis of Water-Soluble Vitamins (B2, B3, B6, B12, and C) and Fat-Soluble Vitamins (E, K, D, A, and β-Carotene) of Okra. J. Chem., 2014:6. doi.org/10.1155/2014/831357

Rucker, R.; Suttie, J.; McCormick, V. and Machlin, L. (2004). Hand Book of Vitamins, 3rd ed. (Marcel Dekker).

Rucker, R.; Suttie, J. ; McCormick, V. and Machlin, L. (2007). Hand Book of Vitamins, 4th ed. (Taylor and Francies Group, LLC).

Schramm, D.; Karim, M.; Schrader, H.; Holt, R.; Cardetti, M. and Keen, C. (2003). Honey with high levels of antioxidants can provide protection to healthy human subjects. J. Agric. Food Chem., 51: 1732.

Semba, R. (2012). The discovery of the vitamins. – NCBI. Int. J. Vitam. Nutr. Res., 82(5): 310-5. doi: 10.1024/0300-9831/a000124.

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تقدير الفيتامينات الذائبة في الماء في عسل المصري باستخدام الكروماتوغرافيا السائلة عالية الأداء

أحمد محمود فرج[1] ، محمود صبرى رزق[1] ، حمدى عبدالنبى الباسل[2] ، منى حنفى يوسف[2] ، فتحي محمد عبد الحليم[1]

1 - كلية العلوم- جامعة القاهرة

2 - المعهد القومى للتغذية- القاهرة

المستخلص

مما لا شك فيه أن المواد الغذائية تلعب دورا أساسيا في نمو الأنسان حيث تقوم بامداد الجسم بما يحتاجه من الفيتامينات والاملاح المعدنية. ولقد تم أعداد وتحسين العديد من الطرق التحليلية لتحديد كمية الفيتامينات القابلة للذوبان في الماء في ستة انواع مختلفة من العسل المصري وهي زهور الربيع ، البردقوش ، دوارالشمس ، زهور البرسيم ، الحمضيات و حبة البركة . الغرض من هذا البحث هو تطوير للطرق المستخدمة لتقدير بعض الفيتامينات الذائبة في الماء في العسل عن طريق تحسين كل خطوة من خطوات طرق التحليل كالاستخراج ، وإعداد العينات ، والفصل والكشف.

أوضحت هذة الدراسة ان تحسين الطرق الكرماتوجرافية الدقيقة لقياس فيتامين ب في المواد الغذائية عن طريق تحسين كل خطوة من خطوات طرق التحليل كالاستخراج، وإعداد العينات، والفصل والكشف، والتحقق من صحة الطريقة المطورة وتطبيق الطريقة لتحديد إجمالي فيتامين ب. وقد لوحظ أن أعلى مستوى لفيتامين C، B_1، B_2 ، و B_6 في عسل الحمضيات ، عسل دوار الشمس ، عسل الحبة السوداء وعسل زهرة الربيع ، على التوالي. في حين لوحظ أعلى مستوى في الفيتامينات B_3 و B_9 و B_{12}في عسل البردقوش الحلو. ولوحظ أدنى مستوى لفيتامين B_3، B_6 و B_{12} في عسل زهرة البرسيم في حين أن أدنى مستوى في الفيتامينات B_2 و B_9 لوحظ في عسل الحبة السوداء. ولوحظ أدنى مستوى لفيتامين C و B_1 في عسل زهرة الربيع والحمضيات ، على التوالي. وقد خلصت الدراسة الى وجود فرق معنوي في محتوى الفيتامينات الذائبة في الماء حسب نوع العسل المصري.