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Determination of Water-Soluble Vitamins in Iraqi Honey Bee and Compare with Others Types by High – Performance Liquid Chromatography

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Abstract

High-performance liquid chromatographic methods are used for the determination of water-soluble vitamins with UV-Vis. Detector. A reversed-phase high-performance liquid chromatographic has been developed for determination of water-soluble vitamins. Identification of compounds was achieved by comparing their retention times and UV spectra with those of standards solution. Separation was performed on a C18 column, using an isocratic 30% (v/v) acetonitril in dionozed water as mobile phase at pH 3.5 and flow rate 1.0m/min. The method provides low detection and quantification limits, good linearity in a large concentration interval and good precision. The detection limits ranged from 0.01 to 0.025µg/ml. The accuracy of the method was tested by measuring average recovery values ranged between 94% - 101 %. For standerd solution, and 93%-99% of honey bee samples.

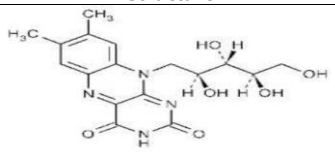
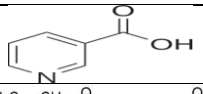
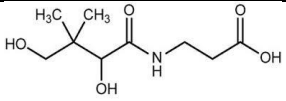
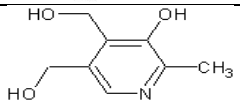
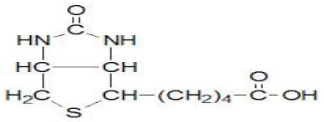
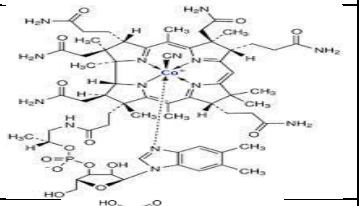
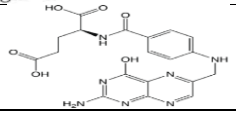
Key words: Iraqi honey, Determination, HPLC.

Introduction

As generally known, vitamins are essential substances, which are necessary for normal health and growth and in sufficient amounts should be supplied by food. If this intake is insufficient or if special dietary requirements exist, multivitamin preparation should be taken in order to prevent vitamin deficiency. Honey is a natural substance produced by bees and is a nutritious food of economic Importance worldwide [1- 3]. Honey is a sweet and viscous fluid created by honeybees from the nectar of flowers [4]. Honey is at most composed of a complex mixture of carbohydrates and other minor substances, such as organic acids, amino acids, proteins, minerals, and vitamins. In roughly all honey types, fructose predominates glucose being the second main sugar. These two account for nearly 85–95% of the honey carbohydrates. More complex sugars made up of two or more molecules of glucose and fructose constitute the residual carbohydrates, except for a trace of polysaccharide. Honey also consists of volatile substances which are

responsible for the characteristic aroma [5, 6].Vitamins are organic substances that are essential in amounts increase activity of the body. They are naturally source from plant and animal foods. The amounts of vitamins ingested from food are measured in micrograms or milligrams [7]. Eight of the water-soluble vitamins are known as the vitamin B-complex group: riboflavin (vitamin B2), niacin (vitamin B3), thiamin (vitamin B1), folate (folic acid), vitamin B6 (pyridoxine), Riboflavin Vitamin B12, biotin and pantothenic acid. The B vitamins are distributed in foods .Water-soluble vitamins are not stored in the body and are easily excreted while fat-soluble vitamins are cumulative in the body and the extravagant assimilation of vitamins A (Retinol) and D (Calciferol) can prove to be harmful [8-10].

Table (1): Chemical Structures of some water soluble vitamins

Vitamins	Structure
Thiamine (B1)	
Riboflavin (B2)	
Niacin (B3)	
Pantothenic acid (B5)	
Pyridoxine (B6)	
Cyanocobalamin (B12)	
Folic acid (B9)	

Materials and Methods

Apparatus

HPLC, Shimadzu LC-10A, (Koyota-Japan). Sensitive Balance, Sartorius, 4digitals, (Germany). pH-meter (Germany).,Ultra sonic bath, Karl Kolb, (Germany). , Magnetic stirrer, various temperature hot plate, UK,Evaporated (Buchi system, Germany).Centrifuge (UK).

Honey samples: honey samples were collected from different regions and market in Iraq as shown in Table (2). Samples were collected in glass bottles and stored in dark prior 25 °C to analysis.

Table (2): Source of honey bees

Honey name	Source
Flower	Arable
Trefoil	Babble
Seder(1)	College of Science of Women
Seder(2)	Alnajef
Eucalyptuses(1)	Alnajef
Nigella sativa	Baghdad
Mountain	Sulaymaniyah
Eucalyptuses(2)	College of Science of Women
Citrus	Baghdad
Eucalyptuses(3)	ALaniber
Olive honey	Southern of Baghdad
Sunflower	West of Baghdad
Germany	Germany
American	American
India	India

Chemicals

All the used chemicals were of the highest purity available analytical grade. Deionized water is used for all purposes. All glass ware, tubes, volumetric flasks, pipettes, tips and other glass were immersed in HNO_3 (5% V/V) for 24 hr. then, rinsed with deionized water. All solvents in this study were grad-HPLC and obtained from BDH, Supelco company (USA) and sigma Company.

Preparation stock Solutions of vitamins

The mixture of soluble vitamins was prepared by dissolving (Thiamine.

Riboflavin, Niacin, Pantothenic, Pyridoxal, Cyanocoblamine) 100µg/ml each was diluted to 25µg/ml**.**

Extraction Samples

One gm of homogenized honey were weighed and dissolved in 1.0 ml of ultra-pure water. Then, 0.1 ml of 2M NaOH diluted (in order to favor the complete solubilization of the honey), and the solution was topped up to mark with ultra pure water in a 25 ml volumetric flask. Sample solutions were injected through a PVDF (13 mm and 0.45 m) .The honey solution was stored in the dark at 4 ◦C until injection[11].

Results and Discussion

Vitamins were essential for human healths which are classified into two groups dissolved in water and soluble in fat. All water soluble vitamins are not stored in the body except B12and B6. These vitamins play an important role in vital functions such as metabolism [12].Honey consists of a mixture of complex such as compounds(flavonoids and phenolic acids, amino acid) which shows various absorption in the UV region compounds, so it is a separation method are most suitable for the extraction of these compounds. We could do by using HPLC method [13].

Optimization of the Separation of vitamins

The Effect of pH on the retention time

The effect of mobile phase pH on in reversed phase HPLC for separation mixture vitamins, to observe the effect of various pH on the retention time of standards. The pH of the system was then varied between ranges of (2.5-7.5), it was adjusted by a few drops of either (0.1 M)HCl or (0.1 M) NaOH solution .The effect of pH changes on the retention time of vitamins show in Table (3).A plot of adjusted retention time (tR) for vitamins , versus pH were present in Figure (1). The optimum pH obtained for best baseline separation of vitamins pH 3.5.

Table (3): Variation retention time (tR) of water soluble vitamins at different pH values.

Vitamin	Retention time (tR min)					
	pH					
	2.5	**3.5**	**4.5**	**5.5**	**6.5**	**7.5**
Thiamine	5.21	3.3	2.10	2.0	1.92	1.013
Riboflavin	6.71	4.3	3.2	2.51	2.1	1.43
Niacin	8.0	5.62	4.13	3.2	2.92	1.51
Pantothenic	9.4	6.6	4.52	3.62	3.21	2.43
Pyridoxal	10.1	8.26	5.61	4.21	3.82	2.62
Cyanocoblamine	13.2	9.06	7.16	6.36	5.12	4.452

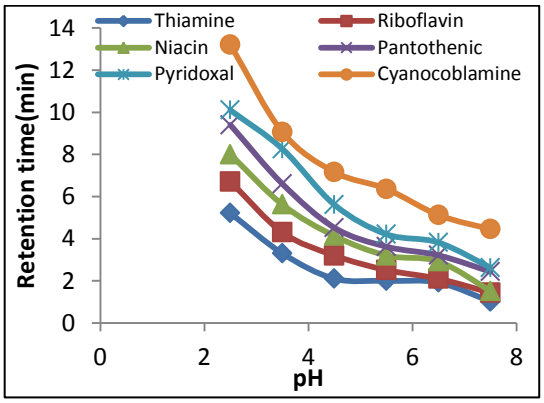


Fig. (1): Plot of adjusted retention time of water soluble vitamin versus pH.

However, it was not possible to cover pH range less than 2.0 and more than 8 due to instability of the packing over this region since the alkaline solution dissolves the silica support and at low pH breaks the Si-O linkage. While at pH region between 5.0-7.0 the retention time is highly changed by the pH variation. This can be attributed to less effective ion-pairing reagent at the higher pH. The pH 3.5 was selected at the best one for vitamins.

Optimization of concentration of Mobile Phase in on elution of water soluble vitamin

In reveres phase methods water is one of the solvents used in the process of and it does not compete with the analyte for the adsorption sites. Washing the column another component of (e.g. acetonitrile), so that usually a modifier because it can interact with the adsorbent surface and compete with analyte molecules for the adsorption sites for dissolve this problem by Increasing the concentration of the modifier in the eluent leads to the decreasing of the analyte retention time. This study was done by using deionized water acidified with and acetonitrile in different ratios. This stage was done by the system of HPLC**.** The results show generally that the retention time was decreased with increasing the % of acetonitrile. This effect has been attributed to a decrease of the surface concentration of the counter-molecule because of the competition by the solvent. Table (4) shows variation of retention time of water soluble vitamins

Table (4): Variation of retention time (tR) of water soluble vitamins on reversed phase at different % acetonitrile flow rate 1 .0ml/min

Vitamin	Retention time (min.)					
	Concentration of acetonitrile (%)					
	5%	10%	20%	30%	40%	50%
Thiamine	8.46	6.25	4.41	3.37	2.21	1.54
Riboflavin	10.6	8.51	6.21	5.81	4.2	3.1
Niacin	11.54	9.26	7.51	6.37	3.89	3.5
Pantothenic	13.4	10.34	8.92	7.6	5.21	4.3
Pyridoxal	14.0	12.7	11.8	10.72	8.21	5.3
Cyanocoblamine	18.4	15.6	13.2	11.68	9.34	7.51

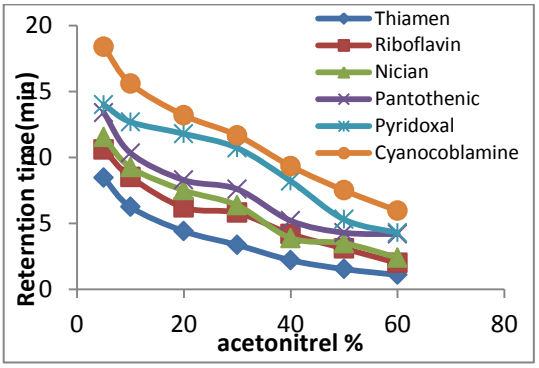


Fig. (2): The Effect of percentage of acetonitrile of separation water soluble vitamins

The Effect of Flow Rate on Separation of water soluble vitamins

HPLC column affected with Increase the flow rate by generating high pressure and reducing the analysis time. So, one must choose a flow rate that is appropriate for HPLC system and column. A higher than usual flow rate may adversely affect the quality of the chromatography not giving the analyte sufficient time to interact with the stationary phase. Faster is not always better. Table (5) and Figure (3) show effect of flowe rate for separation water soluble vitamins. Water Soluble vitamin changed smoothly with a change in flow rate A change in flow rate from (1.6 ml/min to 0.6 ml/min) . Caused a change in the total analysis of time from 5.16 minutes from7.56 minutes to 17.2 minutes for Water Soluble vitamin. 1ml /min gave acceptable separation time for water soluble vitamins.

Table (5): The Effect of flow rate on retention time of water soluble vitamins

Flow rate ml/min	Retention time (min.)					
Vitamin	**0.6**	**0.8**	**1.0**	**1.2**	**1.4**	**1.6**
Thiamine	2.81	3.0	3.37	3.72	4.02	4.51
Riboflavin	4.0	4.92	5.81	6.31	7.51	8.6
Niacin	4.3	5.21	6.37	7.21	8.91	11.2
Pantothenic	4.8	6.51	7.91	9.8	11.7	13.65
Pyridoxal	6.51	8.21	10.76	12.5	14.3	16.1
Cyanocobl amine	17.26	15.31	13.42	11.72	9.72	7.56

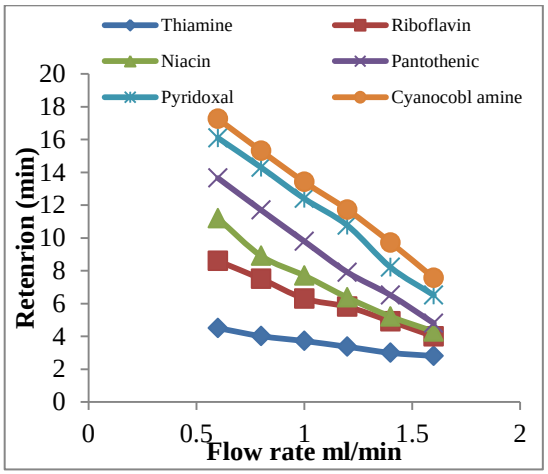


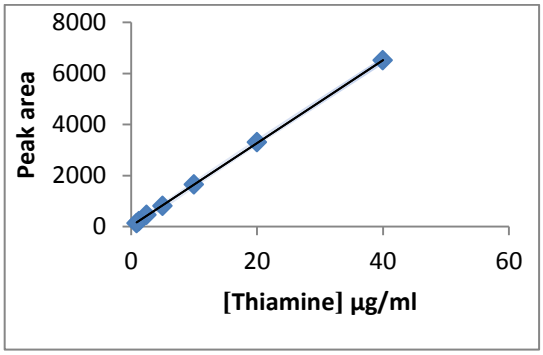
Fig. (3): Retention time as a function of flow rate for Water soluble vitamins.

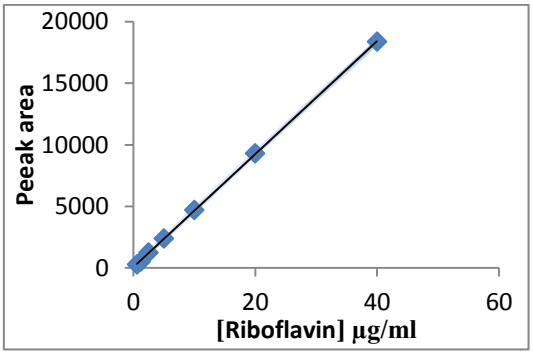
Table (6): The optimum working conditions for the determination of water soluble vitamins

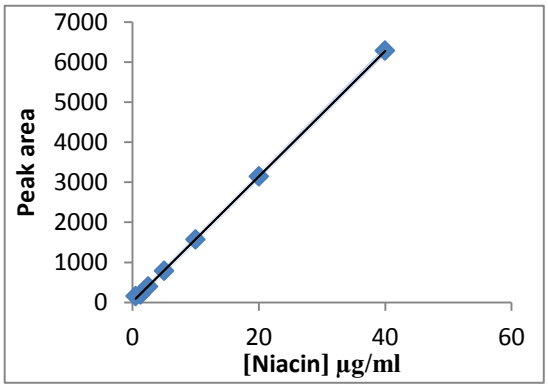
Parameters	Value of water soluble vitamins
Sample volume	20 ↘L
Column	C18(50×4.6mm,i.d)
Organic modifier	30%
pH	(3.5)
Flow rate	1.0 ml/min
↙ maximum	210nm

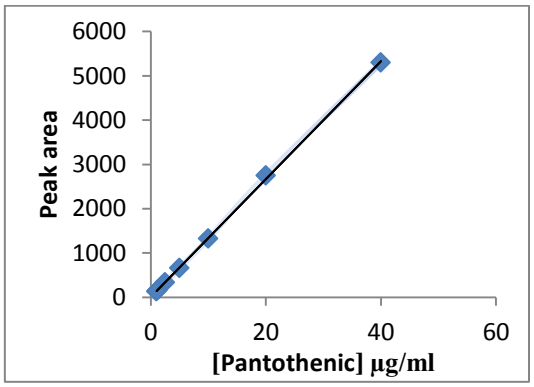
Calibration curve

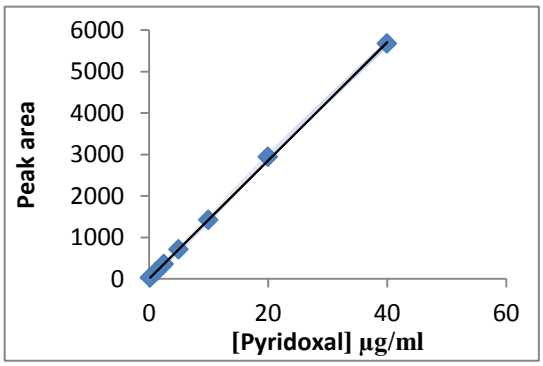
The optimum condition for the separation of amino acid and water soluble vitamin were tabulated in Table (6)











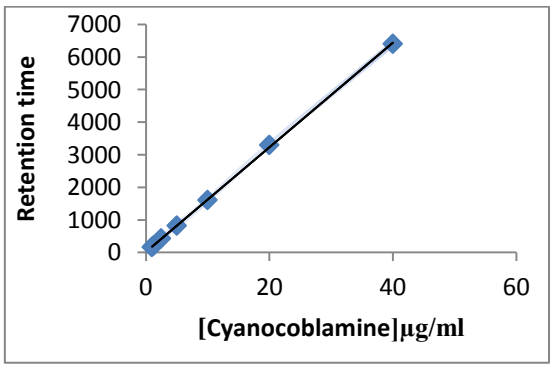


Fig. (4): Calibration curve of water soluble vitamins

Table (8): Error % and Rec % results for analysis of Water soluble vitamins in standard solutions

Vitamins	Conc. of Vitamins µg/ml				
	Present	Found	Error %	Rec %	RSD%
Thiamine	2.00	1.95	2.50	97.50	0.79
	7.00	7.06	-1.00	101.00	0.66
	12.00	11.61	3.30	96.70	0.30
Riboflavin	2.00	2.01	-1.00	101.00	0.24
	7.00	7.13	1.86	102.00	0.83
	12.00	11.85	1.25	98.80	0.32
Niacin	2.00	1.98	1.00	99.00	0.79
	7.00	6.88	1.70	98.30	0.92
	12.00	11.75	2.08	97.92	0.35
Pantothenic	2.00	1.92	4.00	96.00	0.97
	7.00	7.18	-3.00	103.00	0.28
	12.00	11.75	2.08	97.92	0.80
Pyridoxal	2.00	1.88	6.00	94.00	0.90
	7.00	6.65	5.00	95.00	0.33
	12.00	12.06	-1.00	101.00	0.21
Cyanocoblamine	2.00	1.93	3.63	96.37	0.84
	7.00	6.87	1.86	98.14	0.29
	12.00	11.90	0.83	99.17	0.56

Table (9): Error % and Rec % results for analysis of Water soluble vitamins in honey samples.

Type of honey	Conc. of vitamins mg/kg						
	Vitamins	B1	B2	B3	B5	B6	B12
Flower	Present	21.00	3.03	------	3.60	3.50	3.10
	Added	3.00	3.00	3.00	3.00	3.00	3.00
	Found	23.58	6.00	-------	6.52	6.25	5.98
	Rec%	98.30	99.50	-------	98.8	96.10	98.03
	Error%	1.17	0.50	-----	1.20	3.90	1.97
Trefoil	Present	4.20	10.90	4.50	1.72	3.85	3.15
	Added	3.00	3.00	3.00	3.00	3.00	3.00
	Found	7.00	13.78	7.35	4.70	6.75	6.10
	Rec%	97.20	99.14	98.00	99.58	98.54	99.19
	Error%	2.80	0.86	2.00	0.42	1.46	0.81
Seder (1)	Present	27.39	3.03	--------	3.60	3.50	3.10
	Added	3.00	3.00	3.00	3.00	3.00	3.00
	Found	30.20	5.97	-------	6.52	6.32	6.00
	Rec%	99.37	99.00	-------	98.80	970	98.37
	Error%	0.63	1.00	----	1.20	2.80	1.63
Seder (2)	Present	2.40	2.60	4.24	7.50	4.40	3.6
	Added	3.00	3.00	3.00	3.00	3.00	3.0
	Found	5.33	5.43	7.10	10.38	7.23	6.41
	Rec%	98.7	97.00	98.07	99.00	97.7	97.12
	Error%	1.30	3.00	1.93	1.10	.29	2.96
Eucalyptuses (1)	Present	3.20	3.10	3.09	3.50	3.5	2.86
	Added	3.00	3.00	3.00	3.00	3.00	3.00
	Found	6.00	6.00	5.96	6.35	6.40	5.80
	Rec%	96.80	98.4	98.36	97.30	98.5	97.97
	Error%	3.20	1.64	1.66	2.30	1.54	1.03
Nigella sativa	Present	------	5.75	14.50	2.70	4.35	2.59
	Added	3.00	3.00	3.00	3.00	3.00	3.00
	Found	------	8.70	17.30	5.68	7.20	5.48
	Rec%	------	99.43	98,86	99.65	98	98.03
	Error%	-----	0.57	1.10	0.35	2.0	1.97
Mountain	Present	-----	1.30	2.40	6.80	4.79	4.0
	Added	3.0	3.0	3.0	3.0	3.0	3.0
	Found	----	4.28	5.3	9.65	7.5	6.80
	Rec%	-----	99.53	98.2	98.15	96.3	97.1
	Error%	------	0.47	1.9	1.55	3.7	2.9
Eucalyptuses (2)	Present	2.35	2.30	4.7	2.4	2.17	2.5
	Added	3.0	3.0	3.0	3.0	3.0	3.0
	Found	5.3	5.25	7.61	5.31	5.1	5.33
	Rec%	99.07	99.06	98.83	98.33	98.64	96.91
	Error%	1.3	0.94	1.18	1.67	1.37	3.19
Citrus	Present	-------	4.40	4.7	6.1	3.83	3.52
	Added	3.0	3.0	3.0	3.0	3.0	3.0

	Found	-------	7.12	7.6	9.03	6.78	6.33
	Rec%	------	96.1	98.7	99.23	99.27	97.09
	Error%	-----	3.9	1.3	0.77	0.73	2.91
Eucalyptuses (3)	Present	4.50	1.15	6.15	11.3	3.9	4.2
	Added	3.0	3.0	3.0	3.0	3.0	3.0
	Found	7.23	4.10	9.0	14.0	6.78	7.10
	Rec%	96.40	96.00	98.36	97.9	98.26	98.61
	Error%	3.60	1.20	1.67	2.10	1.74	1.39
Olive	Present	------	6.85	2.27	1.83	2.0	1.80
	Added	3.0	3.0	3.0	3.0	3.0	3.0
	Found	------	9.79	5.18	4.8	4.9	4.72
	Rec%	------	99.4	98.29	99.37	98	98.33
	Error%	-----	0.60	1.71	0.63	2.0	1.67
Sunflower	Present	-----	1.30	16.40	1.25	5.16	1.7
	Added	0.3	3.0	3.0	3.0	3.0	3.0
	Found	-----	4.22	19.30	4.20	8.10	4.66
	Rec%	-----	98.13	99.48	98.82	99.26	99.15
	Error%	----	1.86	0.52	1.18	0.74	0.85
Germany	Present	-----	5.80	2.85	6.5	3.0	5.0
	Added	3.0	3.0	3.0	3.0	3.0	3.0
	Found	-----	8.70	5.80	9.4	5.89	7.72
	Rec%	----	98.86	99.14	98.9	98.17	96.5
	Error%	-----	1.14	0.86	1.1	1.83	3.5
American	Present	-----	8.1	6.32	6.54	1.64	5.35
	Added	3.0	3.0	3.0	3.0	3.0	3.0
	Found	----	11.05	9.12	9.33	4.6	8.30
	Rec%	-----	99.55	97.85	97.79	99.14	99.4
	Error%	-----	0.45	2.19	2.25	0.86	0.6
India	Present	21.30	1.50	5.40	5.90	10.75	5.30
	Added	3.0	3.0	3.0	3.0	3.0	3.0
	Found	24.11	4.46	8.2	8.77	13.60	8.26
	Rec%	99.21	99.11	97.61	98.54	98.9	99.52
	Error%	0.78	0.89	2.4	1.46	1.1	0.48

Resolution Measurement

Column performance traditionally has been defined by its reproducibility. Height equivalent to the theoretical plates, HETP (H) and number of plates although useful., but these parameters do not provide sufficient information for properly evaluating column usefulness, capacity factor $\overline{K}$ was also recommended for column evaluation[14]. The results listed in Table (10) show the column parameters for an optimum separation of water soluble vitamins

Table (10): Retention times, capacity factors, and separation factors shows optimum condition for separation of water soluble vitamins

Compound	tR min	$\overline{K}$	⇨	N	R
Thiamine	3.900	2.900	------	521.34	---------
Riboflavin	4.355	3.355	1.2	2626	0.16
Niacin	5.628	4.630	1.4	4387	1.54
Pantothenic	6.606	5.610	1.2	6044	2.75
Pyridoxal	8.265	7.265	1.3	18078	2.09
Cyanocob lamine	9.069	8.100	1.12	1139	0.804

The partition ratio $\overline{K}$, which is commonly called the capacity factor. The capacity factor $\overline{K}$ for water soluble vitamin chromatographic on ODS column were ranged (2.9-8.265) for the analyzed samples as listed in Table (10).The column selectivity, originally called the separation factor ⇨ is defined as the ratio of the capacity factors of two adjacent peaks ⇨= $\overline{K}_2/\overline{K}_1$(The ⇨ represents the separation factor or selectivity representing capacity factor between the two components ⇨ values for water soluble vitamins (1.2-1.4) as listed in Table (10) When the value of ⇨ 1 or more, that means we obtained base line separation for all the eluted mixture. Therefor the optimum separation condition we got, gave mixture under this study, The phase separation mechanism in ODS column depends on the hydrophobic binding interaction between the R group of organic compounds and immobilized

hydrophobic ligand of the stationary phase .The different values of retention times due to interaction between different R groups of organic compound and octadecyle group of the stationary phase governed the mechanism of the retention time. This decreased hydrophilic or hydrophobic interaction with the stationary [14].

Application of the Optimum Conditions for the separation and determination of Water Soluble Vitamin

The analysis of water soluble vitamins in variation honey samples was applied under the optimum condition. The optimum conditions for the separation of water soluble vitamins were obtained by variation in the organic modifier, pH and flow rate. The experimental results of these studies observed the optimum conditions, which gave a base line separation for the whole mixture 30% acetontrile water soluble vitamins respectively , pH 3.5 and flow rate 1ml /min for ,as shown in typical chromatogram Figures (5) and (6)

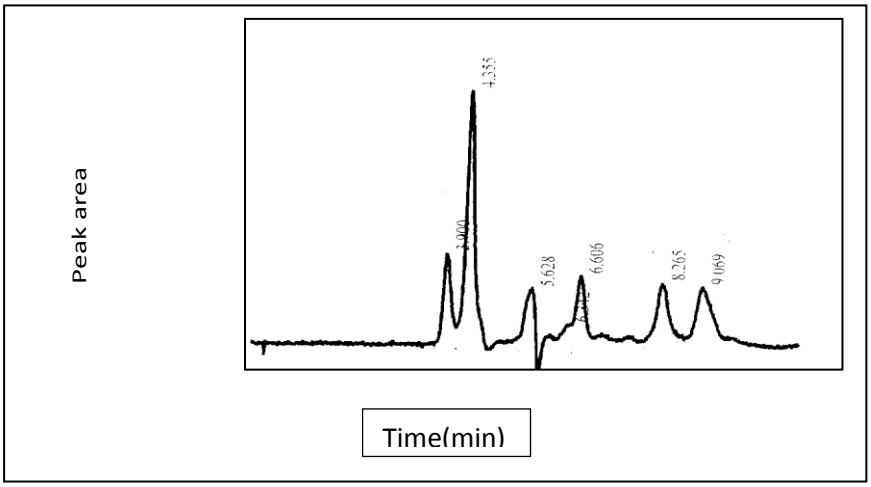
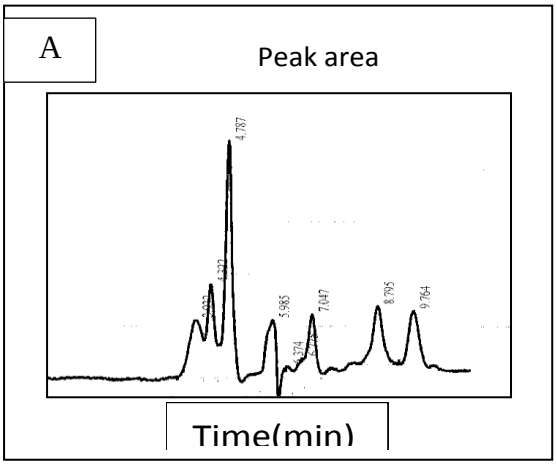
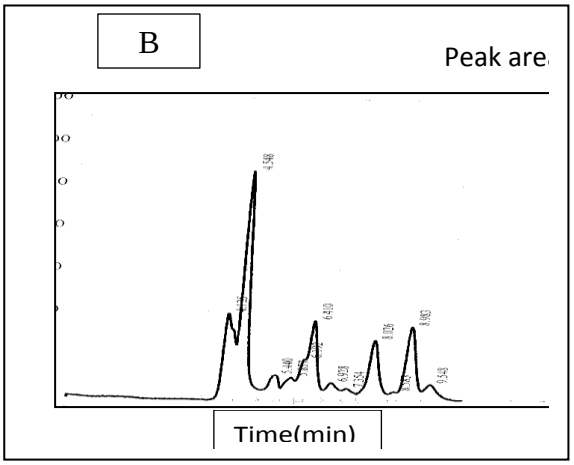


Fig. (5): standard mixture of water soluble vitamins on reversed phase column (50×4.6mm.) using 30% flow 1.0ml/min UV detector 210 nm.





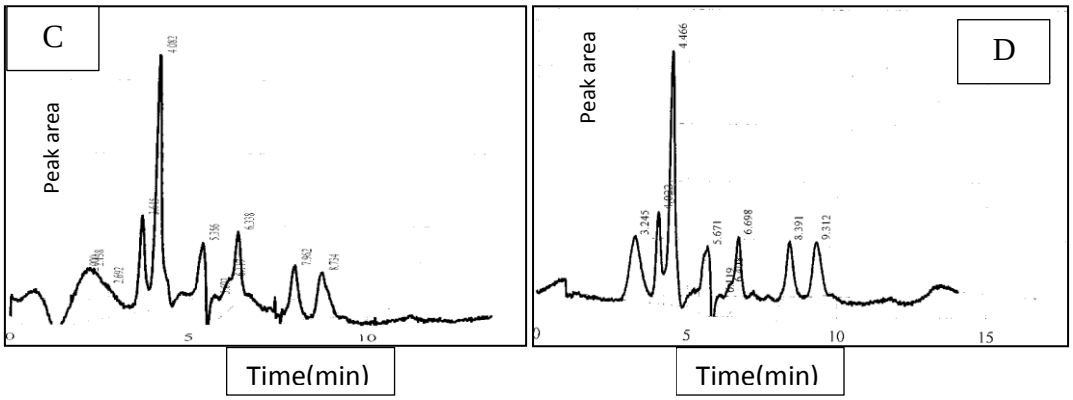


Fig. (6): Separation mixture of water soluble vitamins in Honey samples on reversed phase column (50×4.6mm.) using 30% acetonitrile flow 1.0ml/min UV.

Table (11): Concentration of Water soluble vitamins compounds in honey bee under study values are expressed in µg/100gm as mean ±Sd detector 210 nm(A= Trefoil, B= Mountain (3),C Sunflower ,D= Seder(2))

Type of honey	B1	B2	B3	B5	B6	B12
Flower	21.0±1.000	3.033±1.528	------	3.61±1.000	3.59±1.000	3.14±1.000
Trefoil	4.30±1.528	10.97±1.528	4.48±1.528	1.75±1.5275	3.897±2.08	3.18±1.528
Seder(1)	27.93±1.528	3.047±1.528	3.917±1.528	2.91±1.000	2.77±1.000	2.69±1.000
Seder(2)	2.40±2.000	2.62±2.00	2.598±1.528	7.60±2.000	4.40±1.53	3.61±1.000
Eucalyptuses (1)	3.31±1.000	3.09±1.000	4.26±1.528	3.52.33±1.16	3.55±2.646	2.86±1.528
Nigella sativa	------	5.77±1.000	14.77±1.528	2.72±2.0817	4.36±1.526	2.50±2.000
Mountain	-----	1.303±1.528	2.42±1.5275	6.82±2.517	4.79±2.52	3.97±2.517
Eucalyptuses (2)	2.36±1.528	2.30±2.517	3.91±2.082	2.42±l.000	2.17±2.517	2.50±1.000
Citrus	-----	4.437±3.215	4.68±2.646	6.10±1.528	3.83±4.042	3.49±1.155
Eucalyptuses(3)	4.51±2.081	1.12±2.517	6.14±3.606	11.33±1.528	3.91±1.528	4.20±1.528
Olive	-----	6.857±3.055	2.26±2.646	1.807±2.08	2.00±1.000	1.81±0.876
Sunflower	-----	1.303±1.528	16.42±2.646	1.27±2.0817	5.16±1.528	2.5 3±1.15
Germany	-----	5.81±1.000	2.86±3.215	6.50±1.000	3.01±1.528	5.00±5.508
American	-----	8.12±1.528	6.32±2.082	6.54±4.042	1.64±1.000	5.37±0.674
India	21.36±1.528	1.54±1.528	5.40±1.000	5.90±1.000	10.78±0.054	5.34±1.000

Water -soluble vitamins were measured in different types of honey sample from Iraqi markets which showed various concentrations in table (11) the highest level for B1 ,B2,B3 ,B5,B6,B12 (in Seder(1) honey 27.93 mg/kg, Trefoil honey 10.97mg/kg, Sunflower honey 16.45 mg/kg, Eucalyptuses(3)honey 11.33mg/kg ,Olive honey 2.0 mg/kg, Sunflower2.53 µg/100gm) and lowest level in (Seder(2) honey 2.4 mg/kg, Eucalyptuses(3)honey 1.12 mg/kg, Olive honey 2.26mg/kg, Sunflower honey 1.27 mg/kg, American honey 16.4,Olive honey 1.81 µg/100gm) . We can compare different types of honey samples which found high level for vitamin B2, B3, B5 (920, 278,700) mg/kg in different location [15] and B6 in Brazilin honey 4074 mg/kg [16].

Conclusions

This study was focused to determine Water -soluble vitamins in Iraqi honey samples that produce in University of Baghdad College of Sciences for

Women and other located and compared with other kinds. The obtained results found that it is possible to take advantage of this method that was developed in the set and extract the active compounds in the honey which is water- soluble vitamins of the importance vital. The extraction and separation using a HPLC technique high response, sensitivity and speed too high in separation. Close results and agreement of Iraqi honey samples when compared by extraction methods for honey samples with literature.

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Chromatographic Analysis of Vitamins B1, B2 and B6 in Royal Jelly" J. Braz. Chem. Soc., 15(1): 136-139.

تقدير الفيتامينات القابلة للذوبان في الماء في عسل النحل العراقي ومقارنته مع انواع اخرى باستخدام كروموتغرافيا سائل عالي الاداء

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الخلاصة :

استخدمت كروموتغرافيا لسائل عالي لاداء لتقدير لفيتامينات لقابلة للذوبان في الماء مع كاشف لاشعة فوق لبنفسجية .وقد طورت طريقة لطور العكوس لتحديد لفيتامينات تحت الدراسة . وقد تم تشخيص تلك المركبات عن طريقة مقارنة زمن لاحتجاز للمادة القياسية وباستخدام عمود C18وكانت نسبة لاستونايترال الى الماء لخالي من لايونات 30%(V/V) عند pH3.0وسرعة جريان 1.0 ml/min وقد اظهرت الطريقة دقة وتوافقية جيدة في مدى تراكيز تتراوح (0.01g/ml-0.025-µ) وكانت نسبة لاسترجاع تتراوح بين (94%-101%) للمحلول القياسي وللنماذج المقاسة بين (93%-99%) .

الكلمات المفتاحية :العسل العراقي ،تقدير، HPLC