



Determination of some water- soluble vitamins in different species of garlic extracts by using high performance liquid chromatography

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ABSTRACT

Determination and separation of six water-soluble vitamins including (B₁, B₂, B₃, B₅, B₆, B₁₂) on reversed –phase BDS- C₁₈ (250X4.6 mm id) column were done. Data show that mobile phase containing heptane sulphonate (1.5mM as solvent (A) and mixture of phosphate as buffer B, as ion –pairing reagent, prove excellent separation. The high performance liquid chromatographic method has proved to be rapid and accurate. The results show the highest concentration belongs to B₃ in all types of aqueous garlic extracts but the highest-level concentration is in Iraqi garlic extract (15.58) ppm. The detection limits of Niacin were between ((0.001 –0.125)ppm) for (B₆–B₃, B₁₂) respectively.

Keywords: garlic, vitamin, HPLC.

INTRODUCTION

Many studies have shown the positive dependency of garlic active ingredients with various diseases such as: - Anti-cancer and cancer preventive effects including Inhibiting the growth of bladder tumor [1-4] , Inhibiting the growth of Melanoma Cells (Skin Cancer)[5,6], Inhibiting the growth of prostate cancer cells [7-13], Brain, Neurotrophic, Anti-aging, Anti-depression effects of Aged Garlic Extract (AGE) significantly improve survival, learning behaviors and memory ability. Declines in both cognitive and immune function are predominant features of aging. Improvement of immune function and antioxidative effects were suggested as possible mechanisms for the ameliorating effects of (AGE).

Aged garlic extract also demonstrates neurotrophic effects, an ability to enhance the release of serotonin, an antidepressant effect by Improving Survival, Memory Retention, Learning Deficits and Immune Response[14,15], Enhanced Nerve Growth [16], Enhancement of Human Growth Hormone [17,18], Cardio Protective with Lipid Lowering Effects [19,20], Circulation of Enhancing /Blood-Thinning Properties [21-27].

Aged garlic extract (AGE) has been shown to mitigate infection diseases in humans through enhancement of immune system by Immune Enhancement Effects [28,29] , Inhibition of UV-Induced Immunosuppression [30] and Anti-Allergy Effects [31]. Aged Garlic Extract has demonstrated an array of liver protective effects in studies [32-35].

Vitamins are a diverse group of organic substances occurring in small amounts in all living organisms where they perform many vital functions. Many of them are involved in utilization of the major nutrients like proteins, fats and carbohydrates. Liquid chromatography has been shown to be useful for the simultaneous determination of several

water-soluble vitamins. Separations based on ion-exchange [36], and ion-pair chromatography on C₂ [37], C₈ [37], and C₁₈ columns [38-40] by using 1-hexane sulfonate were reported.

The liquid chromatography analyses of these vitamins have show they are fast (<40 min.) amenable to several water- soluble vitamins simultaneously, and yield accurate quantitative results [38-40]. Traditional methods of vitamin assays have required that each vitamin be determined individually using widely differing physical, chemical, and biological methods. However, fluorimetric (such as Fluorescence intensity) [41], and spectrophotometric methods (such as colormetry) [41] may not provide accurate and precise results for the food matrices tested.

Several excellent HPLC separations of fat-soluble vitamins have been published [42-44] but very few simultaneous and complete characterizations of fat-soluble vitamins in food are available in literature [45]. A reversed-phase HPLC method is described by Blanco D. et al. [46] for the simultaneous determination of vitamins A, D₂, D₃, E, and K₁, retinyl acetate, retinyl palmitate, tocopherol acetate, ergosterol and 7-dehydrocholesterol in milk and butter. Narrow-bore columns are recommended because this alternative provides a good separation and efficiency, plus greater economy and sensitivity.

The aim behind this study is to establish optimized method in liquid chromatography for quantitative evaluation for vitamins and to estimate the effective concentration of active ingredients in garlic for further application.

EXPERIMENTAL SECTION

- Stock Solutions of Vitamins for HPLC: All stock solutions were freshly prepared prior to use(Stock solution of vitamins (B₁, B₂, B₃, B₅, B₆, B₁₂)(100µg/mL)': A weighted amount of (0.1g) standard of thiamin (B₁), Riboflavin (B₂), Niacin (B₃), pantothenic acid (B₅), pyridoxine (B₆) and cobalamin (B₁₂) were dissolved in (30 mL) deionized water containing (60%) methanol. Deionized water was added to bring the volume to (100 mL) in a volumetric flask. An individual wavelength of each compound was detected using ultraviolet spectrophotometer detection Shimadzu 1600.

- Determination of water-soluble vitamins: Vitamins (B₁, B₂, B₃, B₅, B₆, B₁₂) were separated on reversed phase BDS-C₁₈ (250 X 4.6 mm i.d) column with guard column (10 X 4.6 mm i.d), using 0.1 M potassium phosphate buffer pH 2.1 containing 5 mM of 1-heptane sulfonate and acetonitrile (95: 5V/V) as a mobile phase. (50 µl) of the vitamins were injected and the eluted soluble vitamins were detected by UV- detector at (210 nm) with gradient flow rate (2-3) ml/min. (see Table (1)). The limit of detection varies for different vitamins congeners. Higher sensitivity could be obtained at (210nm) with considerable loss in specificity. Calibration of HPLC was made by injection of known concentration sample in the HPLC; the signal generated by the detector was presented as area by a minigrator.

RESULTS AND DISCUSSION

Simultaneous determination of several water- soluble vitamins by liquid chromatography is based on results reported ion- exchange and ion – pair [49-53]. Peak shape, sensitivity, column efficiency, and temperature are the most difficult problem in routine assay of liquid chromatographic analysis.

Ion- pairing reagents that have been used are sodium salts of pentane-, hexane-, or heptane-sulphonic acid, at concentration of c.0.005 M. The position of the water- soluble vitamins peak, relative to other peak in the chromatogram, is determined by the properties of the ion- pairing reagent. One of the ion- pairing reagents, or a combination of them, is usually chosen to ensure that the water- soluble vitamin is adequately separated from interfering substances.

Table (1): Concentration of Water – Soluble Vitamins in Iraqi, Iranian, Lebanese, French, and Chinese garlic extracts

Vitamin	Concentration in garlic extract, ppm				
	Iraqi	Iranian	Lebanese	French	Chinese
B ₁	15.284	12.599	3.424	8.340	8.928
B ₂	13.832	8.300	10.900	10.748	6.753
B ₃	15.576	13.940	12.060	11.404	8.949
B ₅	13.856	7.176	11.204	7.512	6.578
B ₆	13.648	12.316	-	2.788	6.612
B ₁₂	8.320	7.072	9.844	5.928	6.855

Table (2) shows that the concentration of the determined water- soluble vitamins in Iraqi, Iranian, Lebanese, French, and Chinese aqueous garlic extracts is high in (B₃). Vitamin (B₆) is undetectable in Lebanese aqueous garlic extract. In Iraqi aqueous garlic extract shows highest values of determined water- soluble vitamins compared with other aqueous extract, except (B₁₂) in Lebanese extract.

Table (2): The HPLC conditions of water - soluble vitamins (B₁, B₂, B₃, B₅, B₆, B₁₂) analysis

Mobile Phase (A) at pH 2.1 by Phosphoric Acid	((100mM) Anhydrous Sodium Phosphate + (1.5mM) 1- Heptane Sulfonic Acid) 95%
Mobile Phase (B)	5% Acetonitrile (ACN)
Flow Rate ⁽¹⁴⁸⁾	2mL. Min. ⁻¹ for (8 min.) then 3mL.min. ⁻¹ for (15 min.)
Detection: 210 nm	Temperature: 40 °C

Table (3): Standard of water – soluble vitamins

Vitamin	t _R , min.	Conc., ppm	Area
B ₁	4.164	0.9	34029
B ₂	5.793	0.6	59662
B ₃	2.620	1.0	133239
B ₅	10.390	1.2	1514047
B ₆	3.456	0.2	13474
B ₁₂	15.954	1.0	53070

Table (4): Retention time, concentration, and area of water – soluble vitamins in Iraqi garlic extract

Vitamin	t _R , min.	Conc., ppm	Area
B ₁	4.183	15.284	28896
B ₂	5.831	13.832	68773
B ₃	2.648	15.576	103754
B ₅	10.483	13.856	874158
B ₆	3.448	13.648	45978
B ₁₂	15.992	13.216	35067

Table (5): Retention time, concentration, and area of water – soluble vitamins in Iranian garlic extract

Vitamin	t _R , min.	Conc., ppm	Area
B ₁	4.018	12.599	23819
B ₂	5.728	8.300	41260
B ₃	2.582	13.940	92869
B ₅	10.375	7.176	452640
B ₆	3.407	12.310	41467
B ₁₂	16.122	7.072	18762

Table (6): Retention time, concentration, and area of water – soluble vitamins in Lebanese garlic extract

Vitamin	t _R , min.	Conc., ppm	Area
B ₁	4.174	3.424	32373
B ₂	5.839	10.900	54197
B ₃	2.614	12.060	80337
B ₅	10.343	11.204	707714
B ₆	-	-	-
B ₁₂	16.061	9.844	26119

Table (7): Retention time, concentration, and area of water – Soluble vitamins in French garlic extract

Vitamin	t _R , min.	Conc., ppm	Area
B ₁	4.149	8.340	15769
B ₂	5.784	10.748	53442
B ₃	2.615	11.404	75976
B ₅	10.403	7.512	473774
B ₆	3.372	2.788	9390
B ₁₂	16.083	5.928	15728

The optimum separation conditions for analysis of water- soluble vitamins were achieved by using gradient programme. (Table (1)). The results of water- soluble vitamins as a standard, and in samples are tabulated in tables (3-8). The Optimum conditions for separation of standard vitamins were applied. The same conditions were used for separation of Iraqi, Iranian, Lebanese French, and Chinese. Chinese aqueous garlic extract.

Our results indicate K' is between (0.35) to (7.22) by using BDS- C_{18} column. (Table 9). Values of (α) are between (1.46) to (2.23) that indicates a complete separation has occurred. The results in table (9) show that (N) ranges from (237.0) for (B_5) to (5025.23) for (B_{12}). The results indicate that the values of (R_s) varies between (0.9) and (6.23).

Table (8): Retention time, concentration, and area of water – Soluble vitamins in Chinese garlic extract

Vitamin	t_R , min.	Conc., ppm	Area
B_1	4.167	8.928	16879
B_2	5.833	6.753	33573
B_3	2.630	8.949	59619
B_5	10.406	6.578	414953
B_6	3.505	6.612	22273
B_{12}	15.843	6.855	18190

Table (10) shows low detection limits of determined vitamins are [(0.056) ppm, (0.038) ppm, (0.001) ppm, (0.0023) ppm, (0.0125) ppm, and (0.001) ppm] for B_1 , B_2 , B_3 , B_5 , B_6 , and B_{12} respectively.

Table (9): Retention Time(t_R), Capacity Factor(K'), Selectivity (α), Number of Theoretical Plates (N), and Resolution (R_s) of Water – Soluble Vitamins

Vitamin	t_R , min.	K'	α	N	R_s
B_1	4.164	1.14	1.46	492.3	0.90
B_2	5.793	1.99	1.75	443.3	1.50
B_3	2.620	0.35	-	439.3	-
B_5	10.390	4.36	2.19	237.0	1.70
B_6	3.456	0.87	2.23	2330	3.12
B_{12}	15.954	7.22	1.66	1256.94	6.23

Table (10): Detection Limits of Water – Soluble Vitamins(ppm)

		B_1	B_2	B_3	B_5	B_6	B_{12}
Mix A	Conc.	0.90	0.60	1.00	1.20	0.20	1.00
	P.h	1558	1486	8674	37509	1051	53070
Mix B	Conc.	0.45	0.30	0.50	0.60	0.10	0.50
	P.h	769	740	4349	18690	529	26522
Mix C	Conc.	0.225	0.15	0.25	0.30	0.05	0.25
	P.h	381	366	2168	9335	260	13200
Mix D	Conc.	0.113	0.075	0.125	0.15	0.025	0.125
	P.h	178	162	1100	4659	121	6588
Mix R	Conc.	0.0565	0.038	0.063	0.075	0.0125	0.063
	P.h	77	84	547	2319	57	3261
Mix E	Conc.	0.0283	0.019	0.0315	0.038	0.0063	0.031
	P.h	-	-	286	1141	-	1616
Mix F	Conc.	0.0141	0.009	0.0158	0.019	0.0032	0.016
	P.h	-	-	1398	575	-	798
Mix G	Conc.	0.0071	0.0045	0.0078	0.009	0.0015	0.008
	P.h	-	-	680	271	-	384
Mix K	Conc.	0.0035	0.0023	0.0039	0.0045	0.0008	0.004
	P.h	-	-	322	124	-	187
Mix M	Conc.	0.0018	0.0011	0.002	0.0023	0.0004	0.002
	P.h	-	-	153	58	-	88
Mix N	Conc.	0.0008	0.0005	0.001	0.0011	0.0002	0.001
	P.h	-	-	65	-	-	41

P.h: Peak height

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