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DETERMINATION OF L-CARNITINE AND COENZYME Q_{10} ACTIVE SUBSTANCE LEVELS IN FOOD SUPPLEMENTS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

(Penentuan Kadar Zat Aktif L-Carnitine dan Ko-enzim Q_{10} dalam Suplemen Makanan Menggunakan Kromatografi Cecair Prestasi Tinggi)

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Abstract

This study aimed to determine the active substance levels in L-Carnitine and Coenzyme Q_{10} in dietary supplements using the High-Performance Liquid Chromatography (HPLC)-UV method. They are often found in dietary supplements because these ingredients can be used to maintain heart health. L-Carnitine is an active ingredient belonging to amino acids, while Coenzyme Q_{10} is one of the most significant lipid antioxidants, which prevents the formation of free radicals and modification of proteins, lipids, and DNA. The method used in this study is HPLC-UV because the method has good sensitivity, can use the column and solvent developer many times, the detector varies, the analysis time is generally short, easy to operate, and has relatively high accuracy and precision. The HPLC procedure for determining L-Carnitine used a UV detector with a wavelength of 225 nm using an L1 (4.6 mm x 15 cm; 5 m) column with a flow rate of 0.7 mL/min. Meanwhile, the determination of Coenzyme Q10 using the HPLC procedure used a UV detector with a wavelength of 280 nm using a C_{18} (4.6 mm x 15 cm, 5µm) column with a flow rate of 1.5 mL/minute. This study also reports differences in L-Carnitine and Coenzyme Q10 in dietary supplements (in capsule form) during the mixing and encapsulation processes. The results show the suitability test for the HPLC-UV system in good condition; seen from the %RSD value below 2%. In testing the active substance Coenzyme Q_{10} in the mixing process is 101.07% and encapsulation is 96.77%. While the active substance L-Carnitine in the mixing process is 105.05% and encapsulation is 93.03%. The overall test results fall into the range of requirements, meaning that the product quality is in a good category.

Keywords: l-carnitine, coenzyme Q_{10}, high-performance liquid chromatography, mixing, encapsulation

Abstrak

Penelitian ini untuk mengetahui kadar zat aktif L-Carnitine dan Ko-enzim Q_{10} pada suplemen makanan menggunakan kaedah kromatografi cecair prestasi tinggi (HPLC)-UV. Zat aktif ini sering ditemukan dalam suplemen makanan kerana bahan-bahan ini dapat digunakan untuk menjaga kesihatan jantung. L-Carnitine merupakan bahan aktif yang tergolong ke dalam asid amino, sedangkan Ko-enzim Q_{10} adalah salah satu antioksidan lipid yang paling signifikan, yang mencegah pembentukan radikal bebas dan modifikasi protein, lipid, dan DNA. Kaedah yang digunakan dalam penelitian ini adalah HPLC-UV karena memiliki sensitiviti yang baik, dapat menggunakan turus dan pelarut berkali-kali, pengesan pelbagai, memiliki waktu analisis yang singkat, mudah dioperasikan, dan memiliki ketepatan dan kejituan yang relatif tinggi. Prosedur HPLC untuk penentuan L-Carnitine menggunakan pengesan UV dengan panjang gelombang 225 nm menggunakan turus L1 (4.6 mm x 15 cm; 5 m) dengan kadar aliran 0.7 mL/minit. Sedangkan penentuan Ko-enzim Q_{10} menggunakan prosedur HPLC menggunakan pengesan UV dengan panjang gelombang 280 nm menggunakan turus C_{18} (4.6 mm x 15 cm, 5µm) dengan kadar aliran 1.5 mL/minit. Dalam penelitian ini, kami juga melaporkan perbezaan kadar L-Carnitine dan Koenzim Q_{10} dalam suplemen makanan (dalam bentuk kapsul) selama proses pencampuran dan pengkapsulan. Hasil pengujian menunjukkan bahwa uji kesesuaian sistem HPLC-UV dalam keadaan baik dilihat dari nilai %RSD dibawah 2%. Pada pengujian zat aktif Ko-enzim Q_{10} pada proses pencampuran adalah 101.07% dan pengkapsulan adalah 96.77%. Sedangkan zat aktif L-Carnitine pada proses pencampuran adalah 105.05% dan pengkapsulan adalah 93.03%. Hasil pengujian secara keseluruhan menepati piawaian, memberi maksud produk berada pada kategori baik.

Kata kunci: L-carnitine, ko-enzim Q_{10}, kromatografi cecair prestasi tinggi, pencampuran, pengkapsulan

Introduction

This study aimed to determine the active substance levels in L-Carnitine and Coenzyme Q_{10} in dietary supplements using the High-Performance Liquid Chromatography method. L-Carnitine is a component that is needed by the human body. Where L-Carnitine is an amino acid derivative such as a vitamin, which is an important factor in fatty acid metabolism as an acyltransferase cofactor and in energy production processes, such as interconversion in the regulatory mechanisms of ketogenesis and thermogenesis, and is also used in the treatment of primary and secondary deficiencies, as well as in other diseases [1–3]. L-Carnitine is a zwitterion at pH 3.8. L-Carnitine has a molecular weight of 161.2 g/mol with a solubility of >2500 g/L at a temperature of 20 ºC with the chemical formula $C_7H_{15}NO_3$. The structural formula of L-Carnitine is presented in Figure 1 [4].

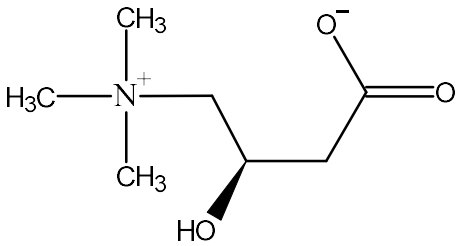

Figure 1. Chemical structure of L-Carnitine [4]

In particular, L-Carnitine among the various forms of Carnitine is also called vitamin B and Carnitine used for food generally means L-Carnitine. L-Carnitine plays a role in helping the breakdown of fat in the human body. Therefore, if it is insufficient, the energy produced in the decomposition of fatty acids is not generated, because the fatty acids do not decompose properly. For this reason, Carnitine is widely used as a drug or food additive to aid diet [5]. L-Carnitine deficiency leads to the accumulation of lipids in the cytosol and impaired energy production of long-chain fatty acids, especially during periods of fasting or stress. L-Carnitine can be found in various foods or medicines such as injections, syrups, tablets, and capsules, which can be used in treating primary and secondary Carnitine deficiency in other diseases such as dyslipoproteinemia and Alzheimer's [6]. Several oral formulations (tablets, capsules, solutions) are commercially available from a wide various of products [7].

The content of L-carnitine in various types of food has been published. Contents vary widely and the highest amounts of L-carnitine are found in meat and dairy products, and a wide variety of fruits and vegetables. In addition to biosynthetic ingredients, L-carnitine can also be found in dietary supplements. Various methods that are sensitive, reliable, relatively simple, inexpensive, and powerful for assessing L-carnitine content are needed to determine the amount of L-carnitine in

various materials [8–10]. Various methods to determine the L-carnitine content in food or other materials have been developed including using high-performance liquid chromatography (HPLC) or gas chromatography (GC), Mass Spectrometric (MS), capillary electrophoresis (CE), TLC-densitometry technique [11–14]. Because L-Carnitine is a polar compound, amphoteric and non-volatile, and does not have a chromophore group [3, 5]. Based on AOAC, the HPLC method can be used to determine L-Carnitine quantitatively [3].

Coenzyme Q_{10} is one of the most significant lipid antioxidants, which prevents forming free radicals and the modifying proteins, lipids, and DNA [15]. Coenzyme Q_{10} is a lipophilic compound consisting of a benzoquinone ring and ten isoprenoid side chain units. [16, 17]. Coenzyme Q_{10} also has poor stability to light and very poor solubility in water. This poor solubility stems from the isoprenoid chain, which is very long in its chemical structure and has a relatively high molecular weight (MW: 864 g/mol) (Figure 2) [18].

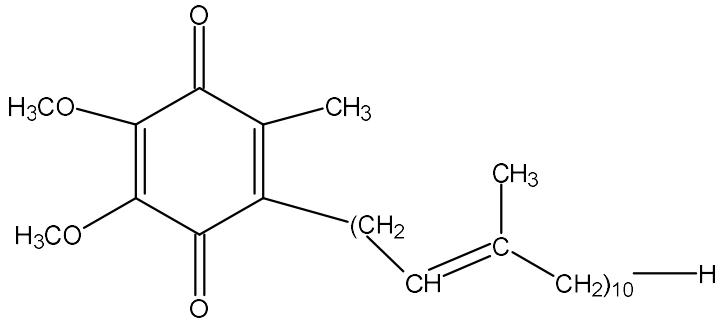

Figure 2. Chemical structure of Coenzyme Q10 [18]

The name Coenzyme Q_{10} comes from its chemical structure, a benzoquinone ring with a side chain of 10 isoprene units. These natural compounds are ubiquitous in nature; thus, it is also known as ubiquinone. There are three types of Coenzyme Q_{10} in the oxidized state the fully reduced form of ubiquinol (CoQ_{10} H_2), the semiquinone radical intermediate (CoQ_{10} H), and the fully oxidized form of ubiquinone (CoQ_{10}) [19–21]. Coenzyme Q_{10} (ubiquinone and its reduced form ubiquinol) can be found in most dairy products, vegetables, fruits, and cereals [22].

Methods of determining Coenzyme Q_{10} in various samples have been carried out such as FT-NIR spectroscopy [23], UPLC/MS-MS [24], ^{1}H NMR Spectroscopy [25], HPLC-ESI-MS/MS [26], HPLC-ECD [27], HPLC with coulometric detection [28], HPLC-UV[17]. Thus, this study discusses the determination of Coenzyme Q_{10} in dietary supplements using HPLC-UV. The HPLC-UV method is an excellent method in all validation parameters (sensitivity, bias, repeatability, intermediate precision, and accuracy profiles) for the determination of Coenzyme Q_{10}. In addition, it allows its widespread use in QC in the pharmaceutical industry [29].

This study reports the determination of L-Carnitine and Coenzyme Q10 in capsules of dietary supplement samples in two manufacturing processes: mixing and filling (encapsulation); the determination will see the levels of L-Carnitine and Coenzyme Q10 from each process. In addition, this test also looks at the precision of the method used which is expressed as Percent Relative Standard Deviation (%RSD).

Materials and Methods

Tools and materials

The tools used in this study include Analytical Balance (Sartorius type B 124 S), Analytical Balance (Shimadzu type ATY124), Disintegrator Tester (Flight type BJ-3), High-Performance Liquid Chromatography (Shimadzu), pH meter (Lovibond type 150), Karl Ficher (Mitsubishi), Spatula, Pro pipette (D&N), Syringe (one road), 0.20 m filter (Phenomenex), 0.4 m filter

(Meissner), Vacuum filter (Duran), Sonification (Elma type S 30 H), Stopwatch (Toto). In contrast the materials used in this research are Acetonitrile 99.9% (JT Backer), phosphoric acid 85%-87% (JT Backer), water for HPLC (JT Backer), Potassium Dihydrogen Phosphate 99% (KH_2PO_4) (JT Backer), 99.5% tetrahydrofuran (JT Backer), 95% N-Hexane (JT Backer), 99.9% Ethanol (JT Backer), L-Carnitine standard, and Coenzyme Q_{10} standard.

Procedure

The research includes two processes: mixing (mixing the capsule's contents) and encapsulation (combining the capsule's contents with the capsule shell, which then becomes a complete capsule. The test is carried out by determining the level of the active substance contained in the supplement, namely L-Carnitine which is a derivative of the active substance L-Carnitine, and also Coenzyme Q_{10} using the high-performance liquid chromatography (HPLC) method.

Instrumentation and optimization of HPLC

The HPLC instrument before being used for the analysis of L-Carnitine and Coenzyme Q_{10} must be optimized first with the specifications in Table 1.

Table 1. Instrumentation and Optimization of HPLC

Description	Condition of HPLC	
	L-Carnitine	**Coenzyme Q_{10}**
Detector	225 nm (UV)	280 nm (UV)
Column	L1 (4.6 mm x 15 cm; 5 µm)	C18 (4.6 mm x 15 cm, 5µm)
Flow rate	0.7 mL/minute	1.5 mL/minute
Injection volume	20 µL	20 L

Preparation of standard solutions of L-Carnitine and Coenzyme Q_{10}

Standard L-Carnitine weighed as much as 100 mg, put into a 50 mL volumetric flask, added solvent to fill 30 mL, sonicated for 5 minutes, then calibrated with solvent to the meniscus mark and homogenized. The standard solution was pipetted as much as 1 mL, put into a 10 mL volumetric flask, diluted with a solvent to the meniscus mark, and homogenized. The standard solution was filtered using a syringe equipped with a 0.20 L filter and inserted into the vial. Then the standard solution was measured using HPLC at a wavelength of 225 nm. The same method was also carried out to determine the Coenzyme standard solution by changing the type of standard used and measuring using an appropriate wavelength of 280 nm for the Coenzyme standard solution.

Determination of the active substance of L-Carnitine by HPLC-UV:

Mobile phase preparation

The mobile phase was made with a mixture of 0.05 M KH_2PO_4 and acetonitrile (4:6), and the pH of the solution was adjusted using phosphoric acid to pH 4.2. The mixture was filtered using a vacuum equipped with a 0.4 L filter and then sonicated for 15 minutes.

L-carnitine analysis using HPLC

The sample was weighed (capsule contents) equivalent to 20 mg of standard, put into a 100 mL volumetric flask, added solvent to fill 50 mL, sonicated for 15 minutes, then calibrated using a solvent to the meniscus mark and homogenized. The sample solution was filtered using a syringe equipped with a 0.20 µL filter and inserted into the vial. Standard solutions were measured using HPLC at a wavelength of 225 nm. This test is repeated in triples.

Determination of the active substance of Coenzyme Q_{10} with HPLC:

Mobile phase preparation

The mobile phase was made with a mixture of acetonitrile: tetrahydrofuran: water (55:40:5). The mixture was filtered using a vacuum equipped with a 0.4 L filter, then sonicated for 10 minutes.

Coenzyme Q_{10} analysis using HPLC

The sample was weighed (capsule contents) equivalent to 100 mg, put into a 50 mL volumetric flask, added solvent to fill 30 mL, sonicated for 5 minutes, and then calibrated with solvent to the meniscus mark and homogenized. 5 mL of the solution was pipetted, put into a 25 mL volumetric flask, diluted with a solvent to the meniscus mark, and homogenized. The sample solution was filtered using a syringe equipped with a 0.20 L filter and inserted into the vial. Standard solutions of Coenzyme Q_{10} were measured using HPLC at a wavelength of 280 nm

Results and Discussion

HPLC system suitability Analysis on L-Carnitine and Coenzyme Q_{10} samples

The system suitability test was carried out using a single standard for six repeated injections, where the %RSD result obtained had to be less than 2%. This value is acceptable because it is below 2% and can be used for quality control of pharmaceutical preparations [6, 30, 31]. Table 2 shows the results of the suitability test for the Coenzyme Q_{10} active substance system.

Table 2. HPLC system suitability test on samples of L-Carnitine and Coenzyme Q_{10}

Description	L-Carnitine				Coenzyme Q_{10}			
	Retention time		Area		Retention time		Area	
	Mixing	Encapsulation	Mixing	Encapsulation	Mixing	Encapsulation	Mixing	Encapsulation
	(minute)		(uAU)		(minute)		(uAU)	
Average test results	1.933	1.995	4378568	4516281	7.827	7.827	3571486	3571486
SD	0.005	0.001	7630	1933	0.0238	0.024	16721	16721
RSD (%)	0.283	0.055	0.174	0.043	0.304	0.304	0.468	0.468

Table 2 shows that the HPLC system suitability test results for L-Carnitine and Coenzyme Q10 standards can be categorized as good. It can be seen from the value of %RSD below 2%. Therefore, this test is feasible for sample analysis in dietary supplements.

Determination of active substance levels in Coenzyme Q_{10} samples

Table 3 shows that the retention time of the sample in the mixing process is longer than the retention time in the encapsulation process. However, this does not affect the results in the separation process using HPLC. Moreover, Table 4 shows that the measurement results are still within the predetermined limits.

Table 3. The retention time of Coenzyme Q_{10}

Description	Retention Time (minute)	
	Mixing	Encapsulation
Sample 1	7.859	7.541
Sample 2	7.827	7.492
Sample 3	7.771	7.457
Average	7.819	7.497

Table 4. Results of the active substance Coenzyme Q_{10}

Process	Description	Area of Sample (uAU)	Area of Standard (uAU)	Standard Concentration %w/v	Content %w/v
Mixing	Sample 1	3635588	3571486	99.62	101.41
	Sample 2	3520627	3571486	99.62	98.20
	Sample 3	3714669	3571486	99.62	103.61
				Average	101.07
				%RSD	2.69
				CV Horwitz	1.9968
Encapsulation	Sample 1	3564557	3571486	99.62	99.43
	Sample 2	3431624	3571486	99.62	95.72
	Sample 3	3411998	3571486	99.62	95.17
				Average	96.77
				%RSD	2.39
				CV Horwitz	2.0099

% w/v is percent weight/volume

Determination of Coenzyme Q_{10} levels using the HPLC method with a UV detector at a wavelength of 280 nm. The use of a UV detector is because the Coenzyme Q_{10} compound has a chromophore group that can absorb wavelengths in the UV region. In addition, the use of the HPLC method uses an inverted phase where the stationary phase in the form of a C18 column is non-polar and the mobile phase used is polar.

Table 4 shows that the results of testing the Coenzyme Q_{10} level in the sample of dietary supplements fall into the required category; the value must be between 90%-115% [32]. Based on the repetition of the sample 3 times, it shows that the precision value seen based on the %RSD shows more than 2%, meaning that the value does not meet the requirements that have been set. Then, the %RSD value was compared with Horwitz's CV value. Because the %RSD value is good if the %RSD obtained is within the acceptable limit, i.e. %RSD is less than 2/3 CV Horwitz [33]. The determination results indicate that the %RSD value is above the CV Horwitz value. Although the %RSD value is greater than the CV Horwitz value, this does not affect the test. The levels obtained in the HPLC test are still within the required range.

Determination of active substance levels in L-Carnitine samples

The results of the retention time of the active ingredient L-Carnitine in Table 5 show that the sample in the mixing process is shorter (1.927 minutes) than the encapsulation process (1.990 minutes). It occurs in HPLC conditions, especially during the test's mobile and stationary phases. The stationary phase is used repeatedly which causes its performance to decrease over time so that it can affect the retention time of the tests performed.

Table 5. The retention time of L-Carnitine levels

Description	Retention Time (minute)	
	Mixing	Encapsulation
Sample 1	1.927	1.989
Sample 2	1.927	1.986
Sample 3	1.928	1.996
Average	1.927	1.990

Table 6. Results of levels of the active substance L-Carnitine

Process	Description	Area of Sample	Area of Standard	Standard Concentration	Content
		(uAU)	(uAU)	%w/v	%w/v
Mixing	Sample 1	4572792	4378568	100	104.44
	Sample 2	4611470	4378568	100	105.32
	Sample 3	4615174	4378568	100	105.40
				Average	105.05
				%RSD	0.51
				CV Horwitz	1.9852
Encapsulation	Sample 1	4175852	4516281	100	92.46
	Sample 2	4185019	4516281	100	92.67
	Sample 3	4243936	4516281	100	93.97
				Average	93.03
				%RSD	0.88
				CV Horwitz	2.0219

%w/v is percent weight/volume

Table 6 shows that the measurement results of L-Carnitine in the sample using the HPLC method in the mixing or encapsulation process have met the specified requirements; the results are in the range of 90%-115%, i.e. 105.05% w/v in the mixing process, and 93, 03 %w/v on the encapsulation process. The sample's precision is determined by repeatability, measuring three times repetition in each process, either in the mixing or encapsulation process. The measurement results obtained that the %RSD value was below the CV Horwitz value. That is, the results of sample precision in each process are in a good category.

Conclusions

The results of the active substance levels in the mixing process of Coenzyme Q_{10} were 103.61% and L-Carnitine was 105.05%, then in the encapsulation process, the results were Coenzyme Q_{10} of 96.77% and L-Carnitine of 93.03%. The internal specifications of the two active substances that have been determined are the same 90%-115%. Based on the comparison of the results obtained with the specifications, the two active substances contained in the supplement meet the range of requirements, meaning that the quality of the chemical test of the product is in a good category.

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