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Pharmacokinetic Profile of Astaxanthin Nanoemulsion Using HPLC (High-Performance Liquid Chromatography) With Oral Routes

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ABSTRACT

Astaxanthin is a very strong antioxidant of the xanthophyll carotenoid group with very lipophilic properties, so in oral administration, its bioavailability is very low. This study aimed to examine the differences in the pharmacokinetic profile of astaxanthin and astaxanthin nanoemulsion via the oral route using the HPLC method. Research methods include validation methods using HPLC and continued determining pharmacokinetic profiles to determine whether astaxanthin in plasma is validated. The plasma was deproteinized with ethanol: tetrahydrofuran (1: 9), mobile phase Methanol: water milliQ: ethyl acetate (82:8:10 (v/v)), the flow rate is 1.2 mL/min, wavelength 474 nm and the sample measured isocratic. Calibration curves 0.25-10 mg/L,% Recovery 102.92, 103.11 and 102.25,% RSD 1.33, 1.65 and 2. The HPLC method is fast, simple, and can be used for routine analysis of astaxanthin. The results showed that in the astaxanthin nanoemulsion, there was an increasing in C_{max} and AUC0-∞ which affected increasing the bioavailability value. Therefore, it was concluded that the study showed the presence of astaxanthin in nanoemulsions has greater absorption power than pure astaxanthin.

Keywords: Pharmacokinetic profile, Astaxanthin, Validation of the HPLC method, Oral route

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1. Introduction

The objectives and hypothesis of the study should be clearly stated. Astaxanthin is a carotenoid xanthophyll with powerful antioxidants (Fassett & Coombes, 2011). Astaxanthin has higher antioxidant activity when compared to various carotenoids such as lutein, lycopene, and carotene (Naguib, 2000). The limited dissolution of astaxanthin in gastrointestinal fluids is one of the causes of its low bioavailability. Another contributing factor is the saturation of carotenoids to enter the micelles formed by bile salts in the gastrointestinal tract at high doses (Domínguez-Hernández et al., 2016; Fassett, R. G., & Coombes, J. S., 2011).

Nanoemulsions can increase solubility in water, improve permeation due to their adhesive properties, prevent enzymatic degradation in the intestinal wall and liver, and protect against gastrointestinal degradation ~~to~~ increase stability and bioavailability (Ragelle et al., 2012; Reyes-Munguía, Azúara-Nieto, Beristain, Cruz-Sosa, & Vernon-Carter, 2009; Shrewsbury, Bosco, & Uster, 2009; Sozer & Kokini, 2009). The oral route is one of the most widely used because of its easy, practical, and convenient application. (Khan, Boateng, Mitchell, & Trivedi, 2015). The development of astaxanthin in nanoemulsions is expected to increase the solubility, dissolution, absorption, and bioavailability of lipophilic drugs through pharmacokinetic testing and parameter determination.

In humans, the bioavailability of carotenoids is low and varies from 10 to 50% of the administered dose due to their low solubility in digestive tract fluids, leading to poor absorption (Nagao, 2011). Astaxanthin is a highly lipophilic compound with low oral bioavailability due to first-pass metabolism. Low bioavailability means low plasma drug levels (Choi, Kang, Yang, Lee, & Shin, 2011). However, the bioavailability of astaxanthin can be increased by introducing the drug into an emulsion form (Odeberg, Lignell, Pettersson, & Höglund, 2003).

This study was conducted to test the pharmacokinetic profile and determine the bioavailability of pure Astaxanthin with astaxanthin nanoemulsion via the oral route using rabbits and the HPLC (High-Performance Liquid Chromatography) method.

1. Method

2.1. Tools

The tools used in this study were laboratory glassware (Pyrex), oral syringe, stopwatch, analytical balance (Toledo Meter), 1 mL syringe (Onemed), 3 mL syringe (Onemed), heparin tube, vortex (MixMat), centrifugation (PLC-05), Eppendorf tube (Onemed), dropper, micropipette, sonicator (power sonic420), vial injector. HPLC (Waters e2695), UV detector, XTerra® column Sunfire C18 5µm (4.6x150mm). Mobile phase Methanol: water milliQ: ethyl acetate (82:8:10(v/v)). With a flow rate of 1.2 mL/minute at a wavelength of 474 nm, the retention time was 2.90-3.50 minutes, and the sample was measured by the isocratic method.

2.2 Materials

The materials used in this study were standard Astaxanthin 97% (HPLC) from Blakeslea trispora, pure Astaxanthin, astaxanthin nanoemulsion, Acetone (merck), Methanol for HPLC grade (Sigma Aldrich), Water milliQ

(merck), Ethyl Acetate for HPLC grade (Merck), Male Rabbits with a body weight of 1.5 - 2 kg, ethanol, and tetrahydrofuran (Merck).

2.3. Selection of Test Animals and Dosage

The test animals were healthy male rabbits with a body weight of 1.5-2 kg. The number of rabbits used was 16, divided into two groups. A single dose of pure Astaxanthin and astaxanthin nanoemulsion via the oral route was given at 7 mg/1.5 kg BW Rabbit. Before the administration of astaxanthin, the blood sample of the rabbit was determined to be negative control.

2.4. Plasma Preparation

5 mL of blood was taken from the ear (marginal vein) using a syringe and then put into a heparin tube. Then, it was centrifuged for 20 minutes at 1,500 rpm, and the plasma was taken. Plasma was then transferred to an eppendorf tube and stored in a refrigerator (-20^{0}C) (Meor et al., 2012). Blood samples were taken from the ear at intervals of about 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, and 92 hours.

2.5. Preparation of standard solutions and Astaxanthin concentration series

Astaxanthin was dissolved with acetone p.a., and a calibration curve of standard astaxanthin was made and added to rabbit plasma with a concentration between (0.25-10 mg/L).

3. Method Validation

3.1 Linearity

Linearity was evaluated from the 0.25 - 10 mg/L calibration curve. Drug-free rabbit plasma was dissolved in a standard solution of astaxanthin with a concentration of 0.25, 0.75, 1.25, 2.5, 5, 8, and 10 mg/L. The calibration curve was obtained by plotting the peak area of astaxanthin with astaxanthin concentration and then determining the correlation coefficient (r).

3.2. Accuracy and Precision

Determine the accuracy using (% recovery) by making three analyte concentrations with the equation:

$$\% \text{ Recovery} = \frac{CF-CA}{C*A}\ x\ 100 \quad \text{(Eq.1)}$$

Information (Eq.1) :
CF = The total concentration of the sample obtained from the measurement
CA = Actual sample concentration
C*A = Concentration of added analyte

Determining Precision by using % RSD (Relative Standard Deviation) with the equation:

$$\%RSD = \frac{SD}{\bar{x}}\ x\ 100\% \quad \text{(Eq. 2)}$$

Information (Eq. 2) :
SD = Standard Deviation.
$\bar{x}$ = The average level of Astaxanthin in the sample.

2.6.3 Determination of LOD and LOQ

The detection limit (LOD) and the quantification limit (LOQ) are calculated by the following formula:

LOD = 3α / S
LOQ = 10α / S

3.3. Blood Sample Preparation

Plasma samples stored in a refrigerator (-20^{0}C) before analysis were thawed at room temperature. 200 µL of plasma was taken and put into a microcentrifugation eppendorf tube and deproteinized using 200 µL of a mixture of ethanol and tetrahydrofuran (1:9). The mixture was vortexed for 2 min and then centrifuged at 14,000 rpm for 20 min. After being centrifuged, it was put into an injector vial, taken by auto injection of as much as 10 µL into the HPLC, and measured the area under the peak of the chromatogram (Meor et al., 2012).

3.4. Data Analysis

In this experiment, the analysis will be used using Microsoft Excel 2010 to determine pharmacokinetics. Statistical analysis of the AUC0-∞ comparison between groups using One Way ANOVA 95% confidence. Statistical analysis was performed using SPSS version 23.0.

4. Result

4.1. Analysis Method Validation Results

4.1.1. Linearity

Astaxanthin standard curve manufacture using rabbit plasma was measured using HPLC at a wavelength of 474 nm, resulting in a calibration curve of 0.25-10 mg/L, which obtained r2 = 0.995. The results of the standard astaxanthin calibration curve can be seen in Figure 1.

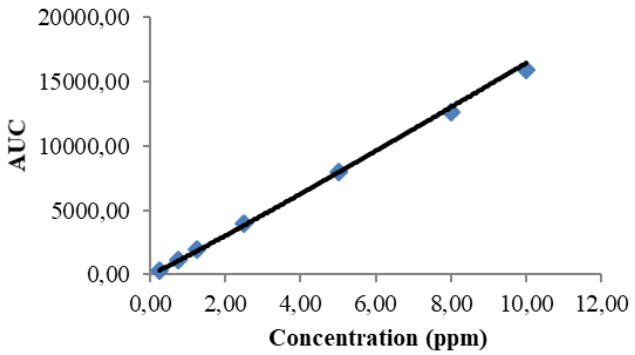

Figure 1. Astaxanthin Standard Calibration Curve Results (Plasma was extracted with standard astaxanthin)

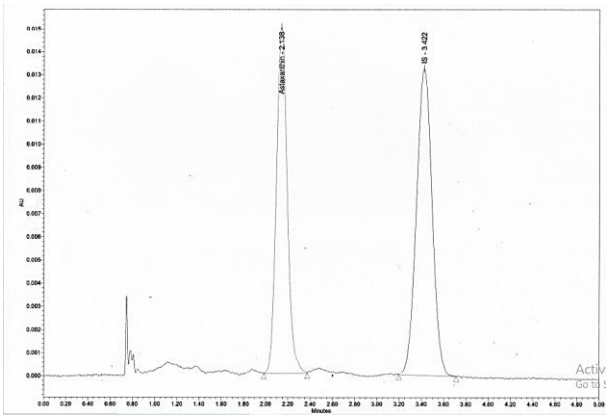

Figure 2. Chromatogram of Astaxanthin under the following conditions: mobile phase Methanol: Water: Ethyl acetate (82: 8: 10 v/v), flow rate 1.2 mL/min, Isocratic method

Accuracy and Precision

The accuracy results show that the replica value from the analysis results is getting closer to the actual sample value with a % Recovery of 102.93, 103.11, and 102.25. These results are still within the range accepted by the FDA, which is 80-120%. The results of precision measurements obtained values of % RSD 1.33, 1.65, and 2. These results meet the FDA criteria of not more than 15%.

3.1.2. Determination of LOD and LOQ

LOD and LOQ measurement results. The value for LOD is 0.08 mg/L, and LOQ is 0.25 mg/L.

4.1.3. Astaxanthin Pharmacokinetic Profile Results

The results of measuring astaxanthin plasma concentrations from 0 hours to 96 hours on pure Astaxanthin and astaxanthin nanoemulsions are presented in Figure 3. Then, the result parameters of the pharmacokinetic profile of Astaxanthin can be seen in Table 1.

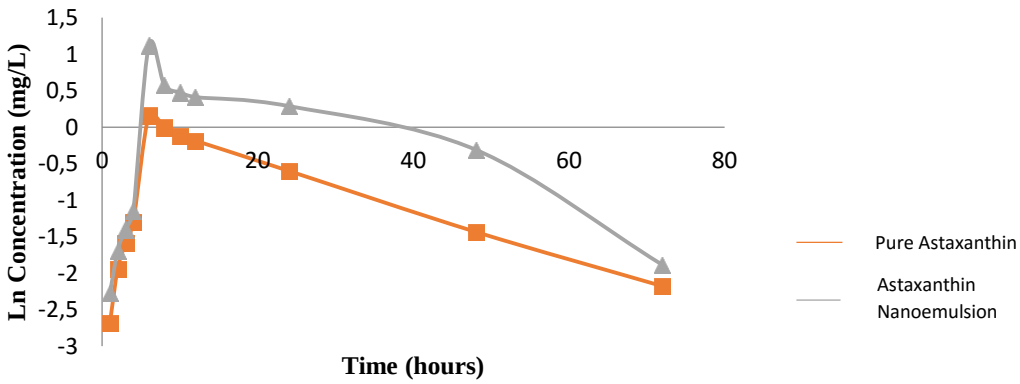

Figure 3. Astaxanthin Plasma Test Results

Table 1. Parameter Results from Pharmacokinetic Profile of Astaxanthin

Pharmacokinetic parameters	Astaxanthin	Astaxanthin nanoemulsion
K_a	0,0638±0,0072	0,9663±0,0199
K_e	0,0309±0,0024	0,0480±0,0019
$_{t1/2abs}$ (hours)	10,95±1,241	0,71±0,0189
$_{t1/2e}$ (hours)	22,53±1,7492	14,50±0,6447
t_{max} (hours)	21,73±2,3619	3,27±0,0896
$AUC_{0-\infty}$ (mg.hours/L)	74,9127±13,2619	95,2615±2,1310
Vd (L)	2,496±0,2496	1,2273±0,0520
Cl (L/hours)	0,0763±0,0145	0,0588±0,0013
C_{max} (mg/L)	1,1723±0,1023	3,9057±0,1585

5. Discussion

The results of the measurement of astaxanthin plasma concentrations from 0 hours to 96 hours on pure Astaxanthin and astaxanthin nanoemulsions are presented in Figure 3 showing the plasma concentration curve of pure Astaxanthin and astaxanthin nanoemulsions showing one-compartment model in the form of monophase (absorption and elimination phases), based on the research conducted by (Odeberg, Lignell, Pettersson, &

Höglund, 2003) on Astaxanthin formulated in lipid-based and (Maltby, Albright, Kennedy, & Higgs, 2003) on Astaxanthin dissolved in sesame oil and gelatin.

Parameter testing of Astaxanthin Pharmacokinetic profiles based on calculations in Table 1 Parameters of absorption speed (K_a) and elimination speed (K_e) in two dosage forms of pure astaxanthin showed faster changes in astaxanthin nanoemulsions with ka values of 0.9663-0.0480. In addition, pure astaxanthin resulted in a $t_{1/2}$ absorption calculation 10.95 hours longer than astaxanthin nanoemulsions.

The value of elimination half-life ($t_{1/2e}$) is inversely proportional to the speed of elimination, so the lower the elimination speed value, the higher the elimination half-life ($t_{1/2e}$) (Katzung, 2011), which means that the time required for astaxanthin to be eliminated is longer. This is shown in pure astaxanthin, and the $t_{1/2}$ elimination calculation is 22.53 hours longer than the astaxanthin nanoemulsion, which is a 14.50-hour $t_{1/2}$ elimination. This does not deviate from the results of previous studies on the pharmacokinetics of astaxanthin, reporting elimination half-lives of 21 hours (Østerlie, Bjerkeng, & Liaaen-Jensen, 2000) and 15.9 hours (Odeberg et al., 2003).

The results showed that astaxanthin nanoemulsions had the highest absorption speed, as indicated by a smaller t_{max} value of 3.27 hours, compared to pure astaxanthin, which was about 21.73 hours. This is because a decrease in the absorption rate indicates that the drug is slowly absorbed by the body, causing a decrease in t_{max}. If the t_{max} value is small at peak times (t_{max}), C_{max} is reached

quickly. However, if the peak time value (t_{max}) is large, the peak level (C_{max}) will be reached for a long time (Shargel et al., 2005).

Based on the calculation results, the peak concentration (C_{max}) is greater in the Astaxanthin nanoemulsion, namely C_{max} 3.9057 mg/L, compared to pure Astaxanthin C_{max} 1.1723 mg/L.

The clearance in the Astaxanthin nanoemulsion was smaller, 0.0588±0.0013 L/hour, followed by pure Astaxanthin at about 0.0763±0.0145 L/hour. The volume of distribution (Vd) is smaller in the Astaxanthin nanoemulsion at 1.2273 L compared to pure Astaxanthin. The relatively high volume of distribution indicates considerable absorption, binding, or metabolism of astaxanthin by extravascular tissues (Østerlie et al., 2000). Statistically, the difference in AUC0-∞ in pure Astaxanthin and Astaxanthin nanoemulsion was significant ($P<0.05$), so the average of each group was significantly different in the dosage form group—the average results of AUC0-∞ in Table 1. The Astaxanthin nanoemulsion is larger, namely 95.2615±2.1310 mg.hour/L, followed by pure Astaxanthin at around 42.8276±0.8739 mg. hour/L. Astaxanthin nanoemulsion showed much greater drug absorption according to research (Meor Mohd Affandi, Julianto, & Majeed, 2012) on Astaxanthin formulated in nanosized and research (Domínguez-Hernández, García-Alvarado, García-Galindo, Salgado-Cervantes, & Beristáin, 2016) on Astaxanthin formulated in the form of nanoemulsions showed that Self-Nanoemulsions increased drug absorption much greater when surfactants were combined with lipophilic and hydrophilic added in the formulation, thereby increasing the surface area of the drug

and increasing its solubility and permeation.

6. Conclusion

Pharmacokinetic Profile Astaxanthin has a significant difference in AUC0-∞ between Astaxanthin nanoemulsion and pure astaxanthin administered orally. Increasing the AUC0-∞ value indicates a much greater drug absorption, affecting the increase in the bioavailability of astaxanthin. The effect of astaxanthin formulation in nanoemulsion can increase the solubility, dissolution, and absorption of lipophilic drugs.

7. References

[1] Ambati RR, Moi PS, Ravi S, Aswathanarayana RG. Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications - A review. Marine Drugs. 2014;12(1):128–152.

[2] Aswinda NMS, Agustina R, Rusli R. Profil Farmakokinetika Simetidin. Proc. Mul. Pharm. Conf. 2015:8–14.

[3] Choi HD, Kang HE, Yang SH, Lee MG, Shin WG. Pharmacokinetics and first-pass metabolism of astaxanthin in rats. British Journal of Nutrition. 2011;105(2):220–227.

[4] Domínguez-Hernandez CR, García-Alvarado MA, García-Galindo HS, Salgado-Cerventes MA, Beristáin CI. Stability, antioxidant activity, and bioavailability of nano-emulsified astaxanthin. Revista Mexicana de Ingeniera Quimica. 2016;15(2):457–468.

[5] Fassett RG, Coombes JS. Astaxanthin: A potential therapeutic agent in cardiovascular disease. Marine Drugs. 2011;9(3):447–465.

[6] Katzung BG. Basic and Clinical Pharmacology. XII. New York: McGraw-Hill. 2011.

[7] Khan S, Boateng JS, Mitchell J, Trivedi V. *Formulation*, Characterisation and Stabilization of Buccal Films for Paediatric Drug Delivery of Omeprazole. AAPS PharmSciTech. 2015;16(4):800-810.

[8] Liu C, Chang D, Zhang X, Sui H, Kong Y, Zhu R, et al. Oral fast-dissolving films containing lutein nanocrystals for improved bioavailability: formulation development, in vitro and in vivo evaluation. AAPS PharmSciTech. 2017;18(8):2957–2964.

[9] Maltby JB, Albright LJ, Kennedy CJ, Higgs DA. Effect of route of administration and carrier on bioavailability and kinetics of astaxanthin in Atlantic salmon Salmo salar L. Aquaculture Research. 2003;34(10):829–838.

[10] Meor M, Abdull A, Julianto T, Majeed ABA. Development of a simple high performance liquid chromatographic method for the determination of astaxanthin in human plasma. Food Science and Technology Research. 2012;18(1):107–113.

[11] Meor M, Abdull A, Julianto T, Majeed ABA. Enhanced oral bioavailability of 1astaxanthin with droplet size reduction. Food Science and Technology Research. 2012;18(4):549–554.

[12] Montenegro-nicolini M, Morales JO. Overview and Future Potential of Buccal Mucoadhesive Films as Drug Delivery Systems for Biologics. AAPS PharmSciTech. 2017;18(1):3-14.

[13] Nagao A. Absorption and metabolism of dietary carotenoids. BioFactors. 2011;37(2):83–87.

[14] Naguib YMA. Antioxidant activities of astaxanthin and related carotenoids. Journal of Agricultural and Food Chemistry. 2000;48(4):1150–1154.

[15] Odeberg JM, Lignell Å, Pettersson A, Höglund P. Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by the incorporation of lipid based formulations. European Journal of Pharmaceutical Sciences. 2003;19(4):299–304.

[16] Paiva SA, Russell RM. Beta-carotene and other carotenoids as antioxidants. J.Am.Coll.Nutr. 1999;18(5):426-433.

[17] Østerlie M, Bjerkeng B, Liaaen-Jensen S. Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin. Journal of Nutritional Biochemistry. 2000;11(10):482–490.

[18] Ragelle H, Crauste-Manciet S, Seguin J, Brossard D, Scherman D, Arnaud P, et al. Nanoemulsion formulation of fisetin improves bioavailability and antitumor activity in mice. International Journal of Pharmaceutics. 2012;427(2):452-459.

[19] Reyes-Munguía A, Azúara-Nieto E, Beristain CI, Cruz-Sosa F, Vernon-Carter EJ. Propiedades antioxidantes del maguey morado (Rhoeo discolor) Purple maguey (Rhoeo discolor) antioxidant properties. CYTA - Journal of Food. 2009;7(3), 209–216.

[20] Shargel L, Wu-Pong S, Yu ABC. Applied biopharmaceutics and pharmacokinetics 5th edition. New York: Appleton & Lange Reviews/McGraw-Hill, Medical Pub. Division. 2005.

[21] Shrewsbury SB, Bosco AP, Uster PS. Pharmacokinetics of a novel submicron budesonide dispersion for nebulized delivery in asthma. International Journal of Pharmaceutics. 2009;365(1–2):12–17.

[22] Sozer N, Kokini JL. Nanotechnology and its applications in the food sector. Trends in Biotechnology. 2009;27(2): 82–89.

[23] Tichý E, Žabka M, Gardavská K, Halenárová A, Kontšeková SZ, Potúčková M. Dissolution and spectrophotometric determination of astaxanthin in aqueous solutions. Pharmazie. 2011;66(8): 560–563.

[24] Vidhyavathi R, Venkatachalam L, Sarada R, Ravishankar GA. Regulation of carotenoid biosynthetic genes expression and carotenoid accumulation in the green alga Haematococcus pluvialis under nutrient stress conditions. Journal of Experimental Botany. 2008;59(6):1409-1418.

[25] Zaripheh S, Erdman JW. Factors that influence the bioavailability of xanthophylls. J. Nutr. 2002;132(3): 531S-534S.