European Journal of Drug Metabolism and Pharmacokinetics
https://doi.org/10.1007/s13318-024-00898-0

ORIGINAL RESEARCH ARTICLE

Comparative Pharmacokinetic Study of Standard Astaxanthin and its Micellar Formulation in Healthy Male Volunteers

Mohamed T. Khayyal[1] · Mahmoud H. Teaima[2,6] · Hoda M. Marzouk[3,6] · Rania M. El -Hazek[4] · Frank Behnam[5] · Dariush Behnam[5]

Accepted: 18 April 2024

Abstract

Background and Objective Astaxanthin is a naturally occurring carotenoid with high anti-oxidant properties, but it is a very lipophilic compound with low oral bioavailability. This study was conducted to compare the pharmacokinetic parameters of a novel astaxanthin preparation based on micellar solubilization technology, NovaSOL® 400-mg capsules (Test product), and those of astaxanthin 400-mg capsules (reference product), after single oral dose administration to healthy male adults.

Methods A single oral dose (400 mg equivalent to 8 mg astaxanthin) of test and reference astaxanthin were administered with 240 mL of water to 12 volunteers according to crossover design, in two phases, with a washout period of 1 week in between. Blood samples were collected at hourly intervals for the first 12 h, then at 24.0, 48.0, and 72.0 h after administration. Aliquots of plasma were centrifuged and the clear supernatant was injected into the high performance liquid chromatography–diode array detection (HPLC-DAD) system. Plasma concentration of astaxanthin versus time profiles were constructed, and the primary pharmacokinetic parameters, maximum concentration (C_{max}), area under concentration time curve from time of administration (0) to time (t) [$AUC_{0\text{-}t}$] or to infinity ∞, [$AUC_{0\text{-}\infty}$], half-life ($T_{½}$) and time to reach C_{max} (T_{max}) were calculated.

Results The test micellar astaxanthin reached a C_{max} of 7.21 µg/ml after 3.67 h compared to only 3.86 µg/ml after 8.5 h for the reference native astaxanthin.

Conclusion Micellar formulation of astaxanthin is capable of producing a high concentration of astaxanthin in plasma in a shorter time, thereby expected to provide faster potential therapeutic efficacy.

Key Points

Through micellation of astaxanthin, its absorption was significantly enhanced.

Increased abosoption of astaxanthin is expected to provide higher therapeutic efficacy.

1 Introduction

Astaxanthin is a naturally occurring carotenoid that belongs to the xanthophyll group, with powerful anti-oxidant activity. The primary and abundant natural source of astaxanthin for commercial use is the microalgae *Haematococcus pluvialis*, synthesized from lycopene or phytoene when exposed to extreme environmental conditions and ultraviolet light

✉ Mohamed T. Khayyal
mtkhayyal@gmail.com

1 Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

2 Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

3 Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo ET-11562, Egypt

4 National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

5 AQUANOVA AG, Darmstadt, Germany

6 Center of Applied Research and advanced Studies (CARAS), Faculty of Pharmacy, Cairo University, Cairo, Egypt

Published online: 15 May 2024

[1]. Astaxanthin has very strong anti-oxidant activity, which exceeds that of other natural anti-oxidants such as vitamin C (ascorbic acid) or vitamin E. Earlier studies have shown that consuming astaxanthin as a daily dietary supplement can lower blood pressure, reduce the risk of stroke, boost immunity, and produce many beneficial metabolic effects [2]. However, astaxanthin, like other carotenoids, is very lipophilic and has low oral bioavailability, a fact that significantly limits its commercial application in the cosmetic and functional food industries [3]. The oral bioavailability of astaxanthin ranges around 10–50% of the given dose, as a result of its poor solubility in water and poor absorption by epithelial cells of the small intestine [4]. One of the first trials to enhance the bioavailability of astaxanthin was carried out by Odeberg et al. [5], who succeeded in increasing its bioavailability by incorporating it into an emulsion form. To increase the water solubility, bioavailability, and potential applications of astaxanthin, various strategies have been investigated, such as formulating it as molecular complexes, microemulsions, liposomes, and solid nanoparticles, but these formulations were not sufficiently effective to make astaxanthin reach the required high blood concentration [6, 7]. Earlier studies have shown that micellar solubilization increases the anti-oxidant activity of xanthohumol [8], as well as that of curcumin and boswellia [9], by increasing their bioavailability. The present study pursued the question of whether micellar solubilization would also result in an increased bioavailability of astaxanthin.

2 Subjects and Methods

2.1 Chemicals and Reagents

Standard astaxanthin (synthetic) US Pharmacopeia, was supplied by Merck (Darmstadt, Germany). Quercetin hydrate 95%, used as internal standard (IS), was obtained from Acros Organics (Belgium, USA). Acetonitrile, methanol, ethanol, tetrahydrofuran, acetic acid, and dichloromethane of HPLC grade were supplied by Sigma Aldrich (St. Louis, MO, USA).

The NovaSOL® capsules test product as well as the native astaxanthin–glycerol preparation, used here as the reference preparation were supplied by AQUANOVA (Darmstadt, Germany), conforming with the regulations of the German Food and Nutrition Authority. They both consisted of 400-mg formulations equivalent to 8 mg of astaxanthin, provided in its native form as an extract from microalgae *Haematococcus pluvialis.*

2.2 Subjects and Study Design

A comparative, crossover, single-dose, two-way, open-label study was designed on 12 healthy male volunteers selected in accordance with the specified inclusion criteria. They were non-smokers, 18–45 years old (mean 39.33 ± 7.02 years) with mean body weight of 82.5 ± 9.05 kg and a body mass index (BMI) ranging from 18.5 to 24.9 kg/m^2 (mean 26.81 ± 1.97) The participants were subjected to complete physical and neurological examination, urine and blood analyses, and gave their written informed consent prior to participation.

Exclusion criteria included the existence of a major medical ailment or hypersensitivity to astaxanthin, history or presence of alcoholism or drug abuse, bleeding tendency, renal or gastrointestinal problems, or participation in a drug research study within the past 3 months.

The study was conducted over two 1-week periods, whereby the volunteers were given one of the preparations during the first period, then allowing a washout period of 1 week before subjecting them to the second preparation. The volunteers were fasted from food for 10 h before the study but were allowed free access to water. They were admitted to the study site in the morning of the first day before drug administration and confined at the site during the period of blood sampling. An indwelling catheter was implanted in the forearm to allow blood sampling (5 mL) at the following intervals following single oral administration of the test astaxanthin (NovaSOL® astaxanthin solubilisate 400 mg equivalent to 8 mg astaxanthin) and reference astaxanthin (native astaxanthin–glycerol preparation 400 mg equivalent to 8 mg astaxanthin) *at* 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 24.0, 48.0 and 72.0 h. The blood samples were taken into pre-labeled tubes containing K_2EDTA as anticoagulant and were immediately centrifuged at 4000 rpm for 10 min. to separate the plasma. The plasma samples were then frozen and stored at – 80°C for further analysis. Sample collection and processing was carried out under conditions with minimal exposure to light.

2.3 Sample Preparation for HPLC Analysis

The plasma samples were thawed at ambient temperature. Aliquots of 50 μL of quercetin (100 μg/ml) as an internal standard (IS) were added to each plasma sample (500 μL) and vortexed for 30 s. The samples were then deproteinized using 1 mL ethanol-tetrahydrofuran mixture (1:9, v/v), then vortexed again for 2 min and then centrifuged for 20 min at 3000 rpm. A volume of 20 μL of the clear supernatant was injected into the high performance liquid

chromatography–diode array detection (HPLC-DAD) system for subsequent analysis.

2.4 Instrumentation and HPLC-DAD Chromatographic Conditions

An HPLC Agilent 1260 Infinity II chromatographic system (Agilent, Waldbronn, Germany) coupled with a DAD quaternary pump system and an auto-sampler was used for quantitative analysis. Data acquisition and processing were achieved using Agilent Technologies open lab CDS 2.x.

Chromatographic separations were performed on a reversed-phase column, Chroma HPLC C_{18} (250 × 4.6 mm, 3.0 μm) column. UV/VIS detection was performed at 480.0 nm. The temperature was maintained at 25 °C with an injection volume of 20 μL and a total run time of 7 min.

2.5 Preparation of Standard Solutions, Calibrators and Quality Control Samples

Primary stock solutions of 200.00 μg/mL of astaxanthin and 100.00 μg/mL IS were prepared separately in glacial acetic acid. Appropriate dilutions were further performed in methanol to obtain different working standard solutions of astaxanthin.

A volume of 50 μL of astaxanthin concentrations were spiked into 450 μL of plasma, so the spiked samples final concentration of calibration standards (CS1-CS9) will be in the range of 1–15 μg/mL for astaxanthin and the quality control (QCs) were 3, 6, and 12 μg/mL for low, medium, and high, respectively.

2.6 Bioanalytical Method Validation

Method validation was performed according to the U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, May (2001) with respect to the following parameters.

2.6.1 Selectivity

Selectivity of the proposed method was evaluated using six different batches of blank human plasma to check for endogenous components, which might interfere with the analyte and IS.

2.6.2 Linearity and Range

Linearity was evaluated by preparing five calibration curves in human plasma, each consists of a blank and calibration standards. Calibration plots were established using peak area ratio of astaxanthin to that of quercetin (IS) versus its corresponding concentration. Linearity was assessed by calculating the linear regression correlation of determination, R^2, over the concentration range of 1.0–15.0 μg/mL and by evaluating the back calculated concentrations of the calibration standards. Concentrations of analyte in QC and study samples were calculated via the linear regression equation. The acceptance criteria were $R^2 \geq 0.9900$ and for calibrators mean % nominal concentrations should be ± 15%, except for the lower limit of quantitation (LLOQ) set at ± 20% and coefficient of variation (CV) % ≤ 15%, except for LLOQ ≤ 20%.

2.6.3 Precision and Accuracy

The intra-day and inter-day accuracy and precision evaluations were performed by repeated analysis of spiked human plasma samples containing different concentrations of astaxanthin on separate occasions. A single run consisted of a calibration curve plus 6 runs of low (LQC), medium (MQC), and high (HQC) quality control samples were analyzed on the same day (n = 6) and on three consecutive different days (n = 18).

The accuracy of the proposed method was expressed through calculating % recovery, whereas precision was judged through CV %. The accepted variation is 15% for all the QC samples except for LLOQ, it was set as ± 20% of the nominal values.

2.6.4 Extraction Recovery

Recovery of astaxanthin was evaluated by comparing analyte responses (peak areas) of two processed sets of LQC, MQC, and HQC quality control samples; one set was spiked before extraction and the other was spiked after extraction. The mean analyte responses of the same concentrations from the two sets were compared. Consistent and reproducible astaxanthin and IS recoveries rather than 100% recoveries are desirable.

2.6.5 Carry-over

A blank sample was injected after the injection of a calibration standard prepared at the upper limit of quantitation (ULOQ). Carry-over in the blank sample following high concentration standard should not be greater than 20% of the peak response of the LLOQ.

2.6.6 Dilution Integrity

Dilution integrity was examined by spiking plasma samples above the ULOQ, at a concentration of 100.0 μg/mL of astaxanthin, then processing in five replicates (n = 5) by tenfold dilution with blank human plasma. Accuracy and precision

should be within the acceptance criteria of 100 % ± 15% and ≤ 15%, in order.

2.6.7 Stability

The stability of astaxanthin in the human plasma matrix was assessed using LQC, MQC, and HQC samples in three replicates (n = 9) at every step of sample preparation, analysis, and storage conditions versus freshly prepared QCs (comparison samples). Short-term stability of astaxanthin in human plasma was tested at room temperature (20–25 °C) for 6 h. For the purpose of evaluating post-operative stability, all quality control levels were submitted to the sample processing procedure and kept at 25 °C (stability samples) for 24 h in the auto-sampler.

2.7 Statistical Analysis

Area under the concentration-time curve (AUC) was calculated using the trapezoidal rule. Statistical differences between treatments were analyzed by one-way ANOVA. A p value of ≤ 0.05 was considered significant. GraphPad Prism version 5.00 for Windows was used for all statistical analyses (GraphPad Software, San Diego, CA, USA). Reported values are arithmetic means with SD or SEM, as indicated.

Primary pharmacokinetic parameters, maximum concentration C_{max}, AUC from time 0 to time t ($AUC_{0\text{-}t}$) and AUC from time 0 to infinity ($AUC_{0\text{-}inf}$) were calculated on the plasma concentration data obtained from bio-analytical laboratory using the PKANALIX Software package (Simulations Plus, USA).

The pharmacokinetic parameters of astaxanthin were estimated using standard non-compartmental methods. The$AUC_{0\text{-}t}$ was calculated from measured data points from the time of administration to the time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule. The $AUC_{0\text{-}inf}$ was calculated according to the following formula:

$AUC_{0\text{-}inf} = AUC_{0\text{-}t} + C_{last} / [\ln(2)/t_{½}]$, where C_{last} is the last quantifiable concentration.

The elimination half-life ($t_{½}$) was calculated as $t_{½} = \ln(2)/(-b)$, where b was obtained as the slope of the linear regression of the ln-transformed plasma concentrations versus time in the terminal period of the plasma curve.

3 Results

3.1 ASTA Determination in Human Plasma Samples

Demographics of 12 study volunteers are presented in Table 1. During the study, no clinically significant abnormalities were observed. In order to extract astaxanthin from the plasma samples of volunteers, they were first deproteinized using an ethanol–tetrahydrofuran mixture (1:9, v/v), which was found to be ideal for providing excellent recovery results. A structurally related quercetin showing comparable physicochemical properties and extraction recovery as astaxanthin was used as IS.

Table 1 Demographics of study volunteers

Volunteer no.	Age (years)	Weight (kg)	Height (m^2)	BMI (kg/m^2)
1	39.00	75.00	1.70	25.95
2	35.00	78.00	1.68	27.64
3	42.00	70.00	1.62	26.67
4	31.00	85.00	1.79	26.53
5	42.00	90.00	1.83	26.87
6	42.00	88.00	1.72	29.75
7	37.00	95.00	1.79	29.65
8	25.00	90.00	1.80	27.78
9	43.00	92.00	1.81	28.08
10	44.00	70.00	1.72	23.66
11	39.00	72.00	1.75	23.51
12	53.00	85.00	1.82	25.66
Mean (SD)	9.33 (7.02)	82.50 (9.05)	1.75 (0.07)	26.81 (1.97)

BMI basal metabolic rate

Optimum performance for HPLC measurement of astaxanthin was displayed using the Chroma HPLC C_{18} (250 × 4.6 mm, 3 μm) column in terms of high resolution, short run time and symmetric peaks. Good peak shapes and high sensitivity were obtained under isocratic conditions using a quaternary mobile phase system composed of methanol–water–acetonitrile–dichloromethane in a ratio of 70:4:13:13, by volume pumped at a flow rate 1.0 mL/min. Ultraviolet/visible (UV/VIS) detection was achieved at 480.0 nm with diode-array detector. The temperature was maintained at 25 °C with an injection volume of 20 μL. Under the optimum chromatographic conditions, the retention times of IS and astaxanthin were found to be 1.9 and 4.8 min, respectively (Fig. 1b).

3.2 Analytical Method Validation

Method selectivity was tested by processing and chromatographing various blank plasma samples prior to use to check for interference from endogenous components. A typical chromatogram is shown in Fig. 1a. No significant interference was observed in any batch of blank plasma at the retention times of astaxanthin and IS.

Linearity was evaluated by preparing five calibration curves in human plasma, each consists of a blank and calibration standards covering the expected range of concentrations (1.0–15.0 μg/mL). Linearity was assessed by calculating the linear regression correlation of determination, R^2, and by evaluating the back calculated concentrations

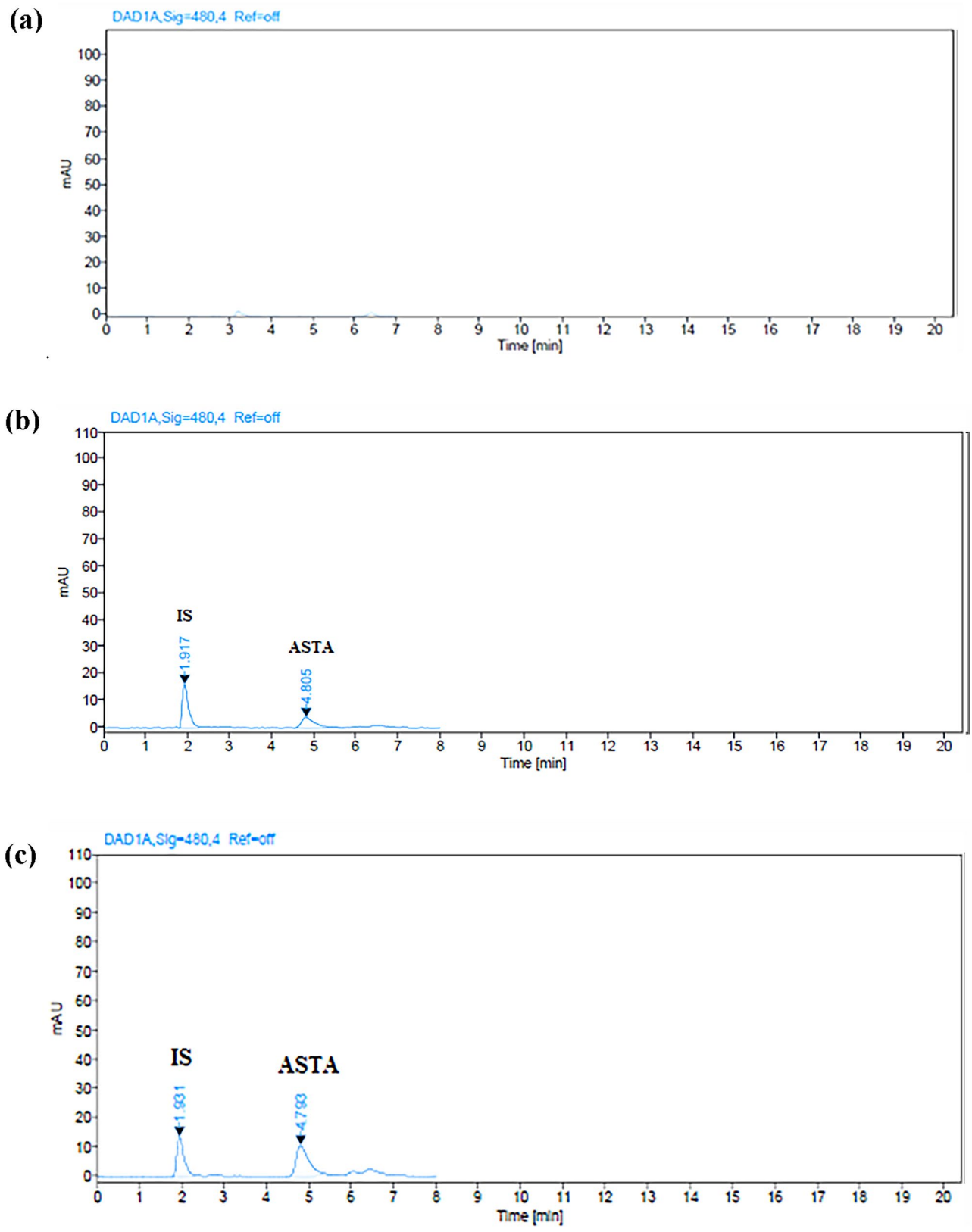

Fig. 1 High performance liquid chromatograms of **a** blank plasma, **b** blank plasma spiked at lower limit of quantitation (LLOQ), and **c** plasma samples of subject at 5 h after oral administration of one capsule (test product) containing astaxanthin (*ASTA*) 8 mg compared to quercetin as internal standard (*IS*)

Table 2 Intra- and inter-day accuracy and precision results for astaxanthin

	Concentration (μg/mL)		Intra-day		Inter-day	
			RE (%)	CV (%)	RE (%)	CV (%)
Analyte	Low QC	3.00	93.23	0.61	98.46	13.13
	Mid QC	6.00	108.22	0.29	107.14	1.23
	High QC	12.00	94.81	3.48	96.44	2.78

QC quality control, *RE* recovery efficiency, *CV* coefficient of variation

Table 3 Stability results for astaxanthin (*ASTA*) in human plasma in different conditions

Analyte	Concentration (μg/mL)		Short-term stability at room temperature (6 h)			Auto-sampler stability at room temperature (24 h)		
			Accuracy (%)	CV (%)	Stability (% change)	Accuracy (%)	CV (%)	Stability (% change)
ASTA	Low QC	1.00	101.40	2.45	− 6.90	94.02	2.64	− 13.67
	Mid QC	3.00	98.14	2.27	− 6.52	98.12	1.74	− 6.53
	High QC	12.00	94.51	1.34	− 5.71	93.63	1.23	− 6.08

QC quality control, *CV* coefficient of variation

of the calibration standards. The LLOQ for astaxanthin is 1.0 μg/mL. Calibration curves were found to be consistently accurate and precise over the calibration range of 1.0–15.0 μg/mL. The mean correlation of determination (R^2) is equal to 0.9995. Back calculations were made from the calibration curves to determine analyte concentration of each calibration standard.

Accuracy of the developed method, expressed as % recovery efficiency, was in the range of 93.23–108.22% and 96.44–107.14% for intra-day and inter-day accuracy, respectively. The CV % for both intra-day and inter-day precision ranged from 0.29 to 13.13%. All these results are summarized in Table 2.

Mean extraction recovery values of astaxanthin were 98.27, 99.06, and 98.84% at LQC, MQC, and HQC levels, respectively. Carry-over was addressed via injecting blank plasma samples after a high concentration sample (ULOQ, 15.0 μg/mL) for astaxanthin. The carry-over in the blank samples was not greater than 20% of LLOQ.

Dilution integrity was checked by diluting spiked human plasma samples at concentration of 100.0 μg/mL, tenfold with blank matrix to bring within the quantitation range by carrying out five determinations per dilution. Precision was expressed as CV% was found to be 1.89% and accuracy result was 107.24%.

Astaxanthin was found to be stable in human plasma at room temperature (20–25 °C) for 6 h. The extracted plasma samples were submitted to the sample processing procedure, the results indicating that samples were stable when stored in the auto-sampler at 25 °C for 24 h. The results are presented in Table 3.

Table 4 Pharmacokinetic parameters of astaxanthin (*ASTA*) following administration of a single oral dose of micellar (*T*) test (NovaSOL® Astaxanthin Solubilisate 400 mg equivalent to 8 mg astaxanthin) and native astaxanthin 400-mg capsule (native astaxanthin–glycerol preparation 400 mg equivalent to 8 mg astaxanthin) delivered to 12 volunteers

Pharmacokinetic parameters	Micellar ASTA	Native ASTA
C_{max} (μg/mL)	7.21 (0.61)*	3.86 (0.33)
T_{max} (h)	3.67 (0.65)*	8.50 (1.38)
$AUC_{0\text{-}t}$ (h × μg/mL)	116.75 (21.11)	104.27 (20.75)
$AUC_{0\text{-}INF}$ (h × μg/mL)	177.06 (24.82)	173.85 (73.53)
$T_{½}$ (h)	32.66 (6.29)	31.76 (16.86)
λ-z (1/h)	0.02 (0.00)	0.03 (0.01)

*$P \leq 0.05$

T_{max} time of the maximum observed plasma concentration, C_{max} maximum observed plasma concentration, $AUC_{0\text{-}t}$ area under the concentration-time curve from time zero to the time t, $AUC_{0-\infty}$ area under curve from time zero to infinity, $T_{½}$ elimination half-life, $\lambda\text{-}z$ rate constant of first order elimination

3.3 Pharmacokinetic Parameters

Primary pharmacokinetic parameters were set to be T_{max}, C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf,}$ and were also considered to be the pharmacokinetic parameter determinants. These parameters were measured following oral administration of test and reference products as shown in Table 4.

The mean plasma concentration-time curves of ASTA with standard deviation bars following oral administration of the test and reference ASTA to the 12 volunteers are

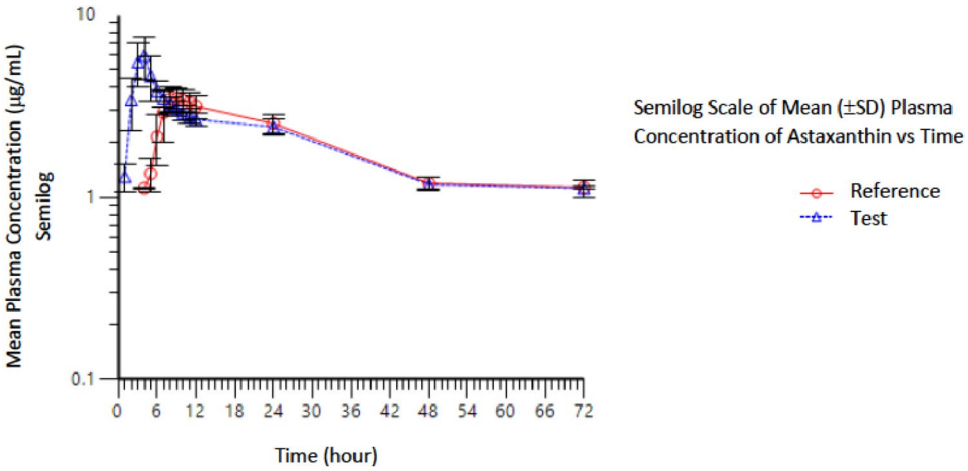

Fig. 2 Graphic presentation on semilog scale of mean (SD) plasma concentration–time curves of astaxanthin following oral administration of test and reference products to 12 volunteers

displayed in Fig. 2. During the study, no clinically significant abnormalities were observed.

The maximum concentration in plasma of the test NovaSol Astaxanthin was 7.21 μg/ml and was reached after 3.67 h after oral administration with a T1/2 of 32.66 h (Table 4). On the other hand, the reference Native Astaxanthin reached its maximum concentration (3.86 μg/ml) in the plasma of patients after 8.5 h with nearly equivalent T1/2 (Table 4).

4 Discussion

Like other carotenoids, astaxanthin has poor aqueous solubility, low absorption rate in vivo, and readily degrades upon exposure to heat, light, or oxygen, thus limiting its application in the field of medicine [10]. Encapsulating astaxanthin into a nano-carrier can increase its solubility and stability, and further expand its application range. At present, the carriers involved in astaxanthin nano-based drug delivery systems mainly include nanoparticles, nanoliposomes, nano-emulsions, nanogels, and nano-micelles [11]. NovaSOL® Nanosome technology from AquaNova German Solubilisate Technologies (AGT) was used in the current study to increase the solubility and bioavailability of poorly soluble astaxanthin by forming nano-sized micelles. Nano-micelles can improve the water solubility of embedded astaxanthin due to the external hydrophilic shell [11]. Astaxanthin can still be absorbed compared to the other nonpolar carotenoids owing to its polar ends in the hydrolyzed form (free form). Its absorption takes place shortly after hydrolysis by cholesterol esterase before it is incorporated into micelles, since astaxanthin is usually presented in the esterified forms (mono- and di-esters) [5]. In the present study, the native astaxanthin in a small dose of 8 mg reached a maximum plasma concentration (C_{max}) of 3.86 μg/mL after (T_{max}) of 8.5 h. On the other hand; it was reported that after oral administration of 100 mg free astaxanthin, the C_{max} and the t_{max} were found to be 1.3 mg/L and 6.7 h, respectively [12]. Similar to the present t_{max}. It has been shown previously that a peak plasma–astaxanthin concentration was reached after receiving a single oral dose of 40 mg [13]. The present study aims to get higher bioavailability of astaxanthin by using micellar technique; a target which was greatly fulfilled where the new formulation of micellar astaxanthin (8 mg) showed a higher C_{max} (7.21 μg/ml) after T_{max} of 3.67 h only. Previous recent trial for astaxanthin but in the form of encapsulated chitosan in a dose of 20 μg/ml was well absorbed in the intestine unlike the free form [14]. The average elimination half-life ($T_{½}$) after a single oral dose of native and micellar astaxanthin was found to be nearly 32 h in healthy volunteers, which means that the new formulation does not affect the elimination of astaxanthin. A similar $T_{½}$ was observed by Okada et al. [15] after a single oral dose of 48 mg astaxanthin in non-smoker volunteers.

The AUCs of native and micellar ASTA after a single oral dose of 8 mg were found to be not significantly different, showing no marked difference imposed by the new formulation. However, the new micellar formula of astaxanthin will be favored due to its faster rate of absorption. After reviewing different forms and doses of astaxanthin in previous studies, the tested astaxanthin dose equivalent to 8 mg of traditional astaxanthin seems to be the smallest effective dose studied. Briefly, when astaxanthin from *Haematococcus pluvialis* algal extract was consumed by healthy adults in a dose of 6 mg/day for 8 weeks, the they showed a normal blood pressure and chemistry indicating its safety [16]. Moreover, astaxanthin at a dose of 16 mg/day (AstaCarox®; AstaReal, Gustavsberg, Sweden) given to 30 men with infertility for 3 months showed a positive effect on sperm parameters and fertility [17]. Higher doses of 40 mg/day of astaxanthin for 4 weeks showed significantly greater reduction of reflux symptoms detected in *H. pylori*-positive

patients [18]. Furthermore, astaxanthin has been used in a clinical trial (Xanthin study) in a dose of 12 mg orally/day for 1 year showing anti-oxidant and anti-inflammatory activity as well as maintaining vascular function in patients who have received a kidney transplant [19].

5 Conclusions

Astaxanthin has significant promise in human nutrition and health but its use has been limited due to low stability and bioavailability. Micellization was effectively used in this pharmacokinetic research utilizing a cross-over design. Micellar formulation of astaxanthin achieved a higher concentration of astaxanthin in plasma in a shorter time compared with the standard formulation, thereby expected to provide faster absorption as well as better potential therapeutic efficacy.

Declarations

Author Contributions All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Funding The study was funded in part by AQUANOVA AG, Darmstadt, Germany.

Data Availability All data supporting the findings of this study are available upon request.

Code Availability Not applicable.

Conflict of interest FB and DB are employed at AQUANOVA AG. The other authors have no relevant financial or non-financial interests to disclose.

Ethical Approval The study was approved by the Ethical Committee for clinical studies set up at the Canter of Applied Research and Advanced Studies (CARAS) at the Faculty of Pharmacy, Cairo University, according to international guidelines in July 2019, approval number BE09071901.

Consent to Participate All volunteers involved in the study gave their consent to participate.

Consent for Publication Not applicable.

References

1. Lorenz RT, Cysewski GR. Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol. 2000;18:160–7. https://doi.org/10.1016/s0167-7799(00)01433-5.
2. Hussein G, Nakagawa T, Goto H, Shimada Y, Matsumoto K, Sankawa U, Watanabe H. Astaxanthin ameliorates features of metabolic syndrome in SHR/NDmcr-cp. Life Sci. 2007;80:522–9. https://doi.org/10.1016/j.lfs.2006.09.041.
3. Wang Q, Zhao Y, Guan L, Zhang Y, Dang Q, Dong P, Li J, Liang X. Preparation of astaxanthin-loaded DNA/chitosan nanoparticles for improved cellular uptake and anti-oxidation capability. Food Chem. 2017;227:9–15. https://doi.org/10.1016/j.foodchem.2017.01.081.
4. Choi HD, Kang HE, Yang SH, Lee MG, Shin WG. Pharmacokinetics and first-pass metabolism of astaxanthin in rats. Br J Nutr. 2011;105:220–7. https://doi.org/10.1017/S0007114510003454.
5. Odeberg JM, Lignell A, Pettersson A, Hoglund P. Oral bioavailability of the anti-oxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. Eur J Pharm Sci. 2003;19:299–304. https://doi.org/10.1016/s0928-0987(03)00135-0.
6. Chen Z, Shu G, Taarji N, Barrow CJ, Nakajima M, Khalid N, Neves MA. Gypenosides as natural emulsifiers for oil-in-water nanoemulsions loaded with astaxanthin: insights of formulation stability and release properties. Food Chem. 2018;261:322–8. https://doi.org/10.1016/j.foodchem.2018.04.054.
7. Li MM, Zahi MR, Yuan QP, Tian FB, Liang H. Preparation and stability of astaxanthin solid lipid nanoparticles based on stearic acid. Eur J Lipid Sci Technol. 2016;118:592–602. https://doi.org/10.1002/ejlt.201400650.
8. Khayyal MT, El-Hazek RM, El-Sabbagh WA, Frank J, Behnam D, Abdel-Tawab M. Micellar solubilization enhances the anti-inflammatory effect of xanthohumol. Phytomedicine. 2020;71:153233. https://doi.org/10.1016/j.phymed.2020.153233.
9. Khayyal MT, El-Hazek RM, El-Sabbagh WA, FranK J, Behnam D, Abdel-Tawab M. Micellar solubilisation enhances the antiinflammatory activities of curcumin and boswellic acids in rats with adjuvant-induced arthritis. Nutrition. 2018;54:189–96. https://doi.org/10.1016/j.nut.2018.03.055.
10. Kim ES, Baek Y, Yoo HJ, Lee JS, Lee HG. Chitosantripolyphosphate nanoparticles prepared by ionic gelation improve the antioxidant activities of ASXaxanthin in the in vitro and in vivo model. Anti-oxidants (Basel). 2022;11(3):479. https://doi.org/10.3390/antiox11030479.
11. Cabral H, Kataoka K. Progress of drug-loaded polymeric micelles into clinical studies. J Control Release. 2014;190:465–76. https://doi.org/10.1016/j.jconrel.2014.06.042.
12. Osterlie MBB, Østerlie M, Bjerkeng B. Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin. J Nutr Biochem. 2000;2863:482–90. https://doi.org/10.1016/s0955-2863(00)00104-2.
13. Ruiz-Núñez B, Schuitemaker GE, Dijck-Brouwer DAJ, Muskiet FAJ. Kinetics of plasma and erythrocyte-astaxanthin in healthy subjects following a single and maintenance oral dose. J Young Pharm. 2014;6(1):42–5. https://doi.org/10.5530/jyp.2014.1.8.
14. Zhu Y, Gu Z, Liao Y, Li S, Xue Y, Firempong MA, Xu Y, Yu J, Smyth HDC, Xu X. Improved intestinal absorption and oral

bioavailability of astaxanthin using poly (Ethylene Glycol)-graft-chitosan nanoparticles: preparation, in vitro evaluation, and pharmacokinetics in rats. J Sci Food Agric. 2022;102:1002–11. https://doi.org/10.1002/jsfa.11435.

15. Okada Y, Ishikura M, Maoka T. Bioavailability of astaxanthin in haematococcus algal extract: the effect of timing of diet and smoking habits. Biosci Biotechnol Biochem. 2009;73(9):1928–32. https://doi.org/10.1271/bbb.90078.
16. Spiller GA, Dewell A. Safety of an astaxanthin-rich *Haematococcus pluvialis* algal extract: a randomized clinical trial. J Med Food. 2003;6(1):51–6. https://doi.org/10.1089/109662003765184741.
17. Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/anti-oxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. Asian J Androl. 2005;7(3):257–62. https://doi.org/10.1111/j.1745-7262.2005.00047.x.
18. Kupcinskas L, Lafolie P, Lignell A, Kiudelis G, Jonaitis L, Adamonis K, Andersen LP, Wadstrom T. Efficacy of the natural antioxidant astaxanthin in the treatment of functional dyspepsia in patients with or without Helicobacter pylori infection: a prospective, randomized, double blind, and placebo-controlled study. Phytomedicine. 2008;15(6–7):391–9. https://doi.org/10.1016/j.phymed.2008.04.004.
19. Fassett RG, Healy H, Driver R, Robertson IK, Geraghty DP, Sharman JE, Coombes JS. Astaxanthin vs. placebo on arterial stiffness, oxidative stress and inflammation in renal transplant patients (Xanthin): a randomised controlled trial. BMC Nephrol. 2008;9:17. https://doi.org/10.1186/1471-2369.