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The enhanced bioavailability of free curcumin and bioactive-metabolite tetrahydrocurcumin from a dispersible, oleoresin-based turmeric formulation

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Abstract

Background: Curcuminoids have been widely studied for human health and disease applications, yet bioavailability remains a hurdle to actualizing all the benefits ascribed to them. The lack of standardization in analysis method, confusion about what constitutes an ideal analyte, and conflicting thoughts around dosing strategies have made it difficult to draw parity between bioavailability and bioactivity and establish a baseline for formulation comparisons.

Methods: This randomized double-blinded, 2-way cross over, single oral dose, comparative bioavailability study differentially evaluates curcumin at the time of its absorption and along various biotransformation pathways, to include free curcumin, the readily usable form of curcumin; individual and composite totals of curcumin and its analogues as exogenously cleaved conjugates, for example, total curcumin, total demethoxycurcumin (DMC), total bisdemethoxycurcumin (BDMC), and total curcuminoids respectively; and the bioactive metabolite of curcumin, total tetrahydrocurcumin (THC). As a primary study objective, the relative bioavailability of CURCUGEN, a novel dispersible, 50% curcuminoids-concentrated turmeric extract was compared to the standard curcumin reference product, curcuminoids 95% standardized extract (C-95), using the maximum concentration (C_{max}), and area under the curve (AUC_{0-t}) of free curcumin, total curcumin, total DMC, total BDMC and the curcumin active metabolite, as total THC.

Results: The evaluation of free curcumin demonstrated that the C_{max} and AUC_{0-t} of the CURCUGEN was 16.1 times and 39 times higher than the C_{max} and AUC_{0-t} of C-95. Furthermore, total curcumin, total DMC, total BDMC, and total curcuminoids resulted in AUC_{0-t} of the CURCUGEN at 49.5-, 43.5-, 46.8-, and 52.5-fold higher than C-95, respectively. The relative bioavailability of CURCUGEN for total THC was found to be 31 times higher when compared to C-95.

Conclusion: As the first human pharmacokinetics study to apply best-practice recommendations and pharmaceutically-aligned guidance in the comprehensive evaluation of a novel curcuminoids formulation, we have established the novelty of said formulation while better standardizing for the common variances and discrepancies between curcuminoids and their derivatives in the literature and commercial marketing, alike.

Abbreviations: AUC = area under the curve, BDMC = bisdemethoxycurcumin, C-95 = curcuminoids 95% standardized extract, C_{max} = maximum concentration, DMC = demethoxycurcumin, THC = tetrahydrocurcumin, T_{max} = time to maximum serum concentration.

Keywords: bioavailability, bisdemethoxycurcumin, CURCUGEN, curcuma longa, free curcumin, tetrahydrocurcumin

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The study protocol was approved by an ethics committee and was conducted in accordance with clinical research guidelines (Clinical Trial Registration Number: CTRI/2019/07/020467).

The full trial protocol can be accessed by contacting the sponsor signatory, Sanjib Kumar Panda, at info@olenelife.com.

Sanjib Kumar Panda and Somashekara Nirvanashetty are employed by Olene Life Sciences Pvt. Ltd. Corresponding author Shavon Jackson-Michel is employed by DolCas Biotech, LLC. These authors were not involved in the analysis of data which was independently conducted by M. Missamma.

The authors report no conflicts of interest.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Turmeric rhizome is the yellow-orange colored cousin of ginger. Ayurveda, the ancient but still-practiced native healing system of India, dates turmeric's medicinal use to as early as 2500 BC.[1] These nearly 5000 year old texts have founded the renown of turmeric today, both in describing its varied therapeutic benefits as well as the need for preparation in maximizing its healing potential. Modern science has revealed that the inherent obstacles, overcome anecdotally through formulation, lie in the low-bioavailability profile of the spice's pigment actives, curcuminoids.[2]

The curcuminoids in turmeric are a collective of individual compounds with very similar structures. The 3 major actives are curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Tetrahydrocurcumin (THC), one of the primary metabolites of curcumin converted in human intestine and liver via enzymatic reduction.[3,4] Curcuminoids have been studied for their various therapeutic applications which include antioxidant, anti-inflammatory, anti-thrombotic, immune stimulatory, cognitive enhancement and anti-cancer activity.[5] Curcumin's anti-inflammatory activity, as demonstrated through scientific studies, is shown to occur by suppressing cyclooxygenase-2, lipoxygenase and inducible nitric oxide synthase via down-regulation of nuclear factor kappa B and other cytokines involved in pro-inflammatory signaling pathways.[5–7] Curcuminoids also display antithrombotic effects, with experiments identifying thromboxane synthesis and arachidonic acid-induced platelet aggregation interferences as probable mechanisms in its inhibitory activity on platelet-activating factor.[8]

Despite curcuminoids diversity of bioactivity, low bioavailability still presents a formidable challenge to realizing its full potential in humans. Formulation strategy, as a compensatory mechanism, has been aimed at one of at least 3 known targets of low-curcuminoid bioavailability: absorption, biotransformation and/or systemic elimination.[9] While not exhaustive, the pharmacokinetic data that does exist for curcuminoids evidences differences in measurable plasma concentrations between diseased and healthy subjects. It also confirms that even high-doses of pure or nearly-pure actives cannot wholly counter the need for formulation as a way to overcome bioavailability constraints. In the first phase 1 clinical trial to evaluate the pharmacokinetics of purified curcumin (99.3%), patients with high-risk or pre-malignant lesions presented with negligible serum levels of curcumin despite receiving doses of 4000 mg/d.[9,10] While patient compliance became an issue at 8 g/d, no treatment related toxicity was found at the max dose of 12 g/d. Contrastingly, a similar dose-escalation pharmacokinetic pilot study evaluating curcuminoids in a healthy population revealed that it took even higher doses than identified in the pre-malignancy population, 10,000 and 12,000 mg of curcumin respectively, to achieve measurable plasma levels.[11]

As purification and high dose strategies have not successfully proven benefit over formulation, novel preparations with varying levels of success in increasing curcuminoids bioavailability have continued to evolve. Combining curcumin with piperine, for instance has increased the bioavailability in rats and human subjects by 150% and 2000%, respectively.[12] Other formulations including emulsions, essential oils complexes and liposomes, reduced particle size through micronization and nanocrystals, or the addition of whey protein, cyclodextrin, and/or surfactants have shown enhanced-bioavailability benefits.[13] Notwithstanding the ingenuity, criticisms have surfaced that question the use and necessity of synthetic adjuncts in some of these formulas, the ratio of formulation ingredients to active material, and/or the limited safety data that exists on these additives or manufacturing processes.[14]

This study purposes to establish the bioavailability advantages of CURCUGEN, a patent-pending curcuminoid formulation, 98.5% derived from turmeric and 50% concentrated in curcuminoids, over a standard reference curcuminoid product, also known as a 95% extract of curcuminoids (or, C-95) in a healthy population. Increased dispersion is a known mechanism of enhancing bioavailability.[15] Turmeric-native polar-resins, which are uniquely co-extracted with the curcuminoids, drive the dispersion characteristics of CURCUGEN and position it for improved absorption kinetics. Of the 98.5% of CURCUGEN turmeric base, 2 notable turmeric-native compounds, including 1.5% of turmeric essential oils and turmeric polysaccharides exist and are posited to complement the formulation's dispersion properties and enhance its bioavailability.[16] As CURCUGEN is not highly purified from the semi-solid oleoresin state wherein curcuminoids, resins and essential oils naturally cohabitate, the curcuminoid analogues: curcumin, DMC and BDMC reflect a uniquely-preserved "food-state" ratio, as characterized exclusively in the turmeric rhizome in its dry-powdered form.[17] The micronized, pharmaceutical/food-grade anti-caking ingredient, silicon dioxide contributes to the formulation's free-flow properties and rounds out the remaining 1.5% of its total composition. Silicon dioxide, trademarked as Aerosil 200 Pharma was sourced from Evonik Industries., India.

Differences in the ratios of major curcuminoid analogues have been proposed as a factor of influence in curcuminoid pharmacokinetics. Research that has investigated the structural differences between the curcuminoids has associated significant clinical[18–22] and pharmacokinetic value to BDMC as the most soluble and stable of the analogues.[23] One study established BDMC as 7× more stable than curcumin, with a duration of stability 3-folds greater than curcumin.[23] It was the conclusion of that study that BDMC's stabilizing role plausibly helps curcumin metabolites arrive to "the right place at the right time"[23] contributing kinetically to curcumin's bioactivity. The concentration of BDMC, as compared to curcumin in dried turmeric rhizome is starkly different from the ratio of BDMC to Curcumin found in C-95.[17] Likewise, CURCUGEN, in its ability to preserve the ratio of this "food-state" delivers from 2 to 7× more BDMC (weight-to-weight basis) than C-95 extracts or the various formulations that derive from it.

Beyond formulation differences, curcuminoid analytical practices – which are foundational to the measurement of plasma curcuminoids concentrations and the bioavailability claims that result, have been described as "scantily detailed and outdated".[14,24] The best practices of bioavailability study design have been described as being inclusive of an established and standardized HPLC method that analyzes for Curcumin, DMC, BDMC, and THC; having a minimum sampling duration of 12 hours post intake with regular testing intervals; and utilizing sufficient quantities of C-95, as an edible, highly purified (i.e., 95%) curcuminoids extract and reference standard to establish measurable baseline blood plasma concentration for the comparison of the test formulation (e.g., CURCUGEN).

Our study utilized a method of determining plasma curcuminoid concentration by LC/MS/MS analysis as previously reported.[25,26] Analytical methods were developed and validated to best appropriate our samples and study goals of including free

curcumin, total curcumin, total DMC, total BDMC and total THC in our evaluations. Of importance, this study appropriated LC-MS-MS as a measurement tool over HPLC, given its resiliency. Described as being less affected by potential matrix interference, LC/MS/MS has been regarded as the ideal method for analyzing curcuminoids.[24] Our study exceeded the minimal sampling duration of 12 hours, with a 24-hour design and dosed curcuminoids in both treatment and reference groups at 2 g, as this was the minimum curcuminoids dose found to have achieved consistently measurable plasma levels in previous studies.[14,27]

2. Methods and materials

2.1. Characteristics of study participants

This study was a double-blind, randomized, balanced, single dose, 2-treatment, 2-sequence, 2-period, 2-way crossover, comparative oral bioavailability study with 7 days' washout period between each study period, under a minimum of 10 hours of fasting conditions.

Healthy adult male participants between 18 and 45 years of age, willing to consume 8 capsules of the investigational products with 300 ± 2 mL of water, over 2 periods were admitted into the trial. Normal health was determined by medical history and physical examination. Subjects who were using any curcumin-containing supplements or foods containing high concentrations of curcumin within 14 days prior to dosing, or who were not willing to take turmeric-free meals for the entire study duration were excluded. Similarly, subjects who were using any OTC products within 14 days or 5 half-lives of the drug prior to dosing, who had a history or presence of significant gastric and/or duodenal ulceration, who were using any recreational drugs, or had a history of drug addiction were excluded. Subjects who consumed xanthine or derivative containing food or beverages, or grapefruit and its juice within 48 and 72 hours respectively, of dosing in each study period and throughout the sampling time points were also excluded.

Selected study volunteers, who were confirmed negative for alcohol and drug abuse by breath alcohol testing and urine drug screen for Benzodiazepines, Cannabinoids, Amphetamines, Cocaine, Barbiturates and Opiates, were admitted to the clinical pharmacology unit, located at Vimta Labs Ltd., India. They were served a turmeric-free dinner on the evening before product administration (i.e., 11:00 hours pre-dose), were fasted for a minimum of 10 hours overnight and were housed at the clinical pharmacology unit for post-dose sample collection 24-hours post dose. Turmeric-free meals/snacks were provided at 4, 8 and 13-hours post dose. As both the CURCUGEN and C-95 groups were both fed the same foods throughout the entire testing period, it is likely that any conceivable benefits asserted by other traditional spices used in the food preparations would be absorbed by the homogeneity of the nutrition protocol.

CURCUGEN is a 50% by HPLC, curcuminoids-concentrated turmeric extract patented by Olene Life Sciences Pvt. Ltd., India. The standard reference curcuminoid product (C-95), a 95% by HPLC-standardized, edible, curcuminoids powder extract – consisting of curcumin, DMC and BDMC, was sourced from Plant Lipids, Pvt. Ltd., India.

A single dose of either treatment was used in this design. Group 1 was given CURCUGEN, containing approximately 2 g of total curcuminoids. Group 2 was given C-95, containing approximately 2 g of total curcuminoids. The equivalent curcuminoid doses were delivered to both groups in 8, 2-part hard gelatin capsules. Instead of using an equation to normalize for formulation differences in the end, we adhered to the FDA Guidance for the Bioavailability and Bioequivalence Studies in maintaining a negligible difference (±5%) between reference and test actives.[28]

Pharmacokinetics outcomes from the evaluation of CURCUGEN in a healthy, male adult population under 10-hour minimum fasting conditions, inclusive of the assessment of free curcumin, total curcumin, total DMC, total BDMC and total THC using analytical and methodological best practices[14] was the primary objective of this study design. Outcomes describing any adverse events (AEs), safety, and tolerance issues derived from data collected during the course of the study were collectively considered the secondary objective of this study.

2.2. Sample collection

Blood samples for the pharmacokinetic evaluation were collected in a staggered fashion within ±2 minutes of the specified sampling time, starting at no less than 11 hours from the turmeric-free dinner meal provided in-house on the evening before. Samples were collected by using intravenous cannula up to 12 hours post dose and the remaining blood samples were collected by means of direct, sterile venipuncture using pre-labeled 6 mL K2EDTA vacutainers. A total of 16 blood samples were collected as per the following schedule in each period:

The pre-dose samples were collected within 01.00 hour prior to study product administration, 00.00 hour and the others at 00.25, 00.50, 01.00, 01.50, 02.00, 02.50, 03.00, 03.50, 04.00, 06.00, 08.00, 12.00, 16.00 and 24.00 hours post dose.

After collection, the blood samples were placed in a Thermocol box containing ice packs. Blood samples were centrifuged at 3800 rpm for 10 minutes at 2 to 8 °C for separating the plasma. Centrifugation of all samples was done within 30 minutes after withdrawing at each sample time point. All plasma samples were separated and were divided into 3 aliquots 1 mL each in properly labeled polypropylene tubes and immediately stored at -70 ± 10 ° C until completion of analysis. The time from sample collection to placement in the freezer did not exceed 120 minutes.

2.3. Sample preparation and analysis for free curcumin

Plasma samples were withdrawn from the freezer and allowed to thaw under monochromatic light at room temperature. A 0.4 mL of plasma was transferred to clean vials and 20 μL of an internal standard, Curcumin-D6 followed by 40 μL of Hydrochloric acid was added and vortexed to ensure the mixing of contents. After the thorough mixing, the curcuminoids were extracted with 2.5 mL of extraction solvent [Ethyl Acetate: Acetonitrile (95:05)] by placing the mixture on a shaker for 10 minutes followed by centrifugation at 4000 rpm at 20 °C for 10 minutes. The supernatant (organic layer) was transferred into another vial and the solvent was evaporated to dryness under a stream of nitrogen at 50 °C. The dried residue was reconstituted in 0.2 mL of mobile phase and 20 μL of the reconstituted sample was injected into an LC-MS/MS system using auto sampler.

The plasma samples were analyzed on a HPLC system with tandem mass spectrophotometry detection (HPLC/MS/MS) using an Agilent Zorbax Eclipse XDB-C18 (3.5 μm, 4.6 × 100 mm) column and the mobile phase of 0.2% Formic Acid in water: Acetonitrile (40:60) at the flow rate of 0.8 mL/min. The column

oven temperature was set to 50°C during the analysis. Free curcumin was quantified against standard curve for Curcumin (purity 99.63%).

2.4. Sample preparation and analysis for total curcumin, total demethoxycurcumin and total bisdemethoxycurcumin

Plasma samples were withdrawn from the freezer and allowed to thaw under monochromatic light at room temperature. A 0.2 mL quantity of plasma was transferred to clean vials and 50 μL of the internal standard Curcumin-D6, followed by 200 μL of 0.08% Formic acid (in 0.1 M KH_2PO_4) was added and vortexed to ensure the mixing of contents. After the thorough mixing, 200 μL of β-glucuronidase/sulfatase (EC 3.2.1.31) from Helix Pomatia in 0.1 M phosphate buffer was added and vortexed. The mixture was incubated for 1 hour at 37°C to ensure the complete hydrolysis of glucuronide/sulfate conjugates of Curcuminoids. Then, the curcuminoids were extracted with 2 mL of extraction solvent [Ethyl Acetate: Acetonitrile (95:05)] by placing the mixture on a shaker for 10 minutes followed by centrifugation at 4000 rpm at 20°C for 10 minutes. The supernatant (organic layer) was transferred into another vial and evaporated to dryness under a stream of nitrogen. The dried residue was reconstituted in 0.5 mL of mobile phase and 20 μL of this reconstituted sample was injected into LC-MS/MS system using auto sampler.

The plasma samples were analyzed on a HPLC system with tandem mass spectrophotometry detection (HPLC/MS/MS) using Kinetex EVO C18 (2.6 μm, 4.6 × 100 mm) column and the mobile phase of Acetonitrile: 10 mM Ammonium Formate (in 0.5% Formic acid in water) at the flow rate of 0.5 mL/min. The column oven temperature was set to 50°C during the analysis. total curcumin, total DMC and total BDMC were quantified against respective standard curve for Curcumin (purity 100%, USP grade), DMC (purity 98%, USP grade) and BDMC (purity 99%, USP grade).

2.5. Sample preparation and analysis for total tetrahydrocurcumin

Plasma samples were withdrawn from the freezer and allowed to thaw under monochromatic light at room temperature. A 0.25 mL of plasma was transferred to clean vials and 50 μL of the internal standard Curcumin-D6, followed by 200 μL of 0.08% Formic acid (in 0.1 M KH_2PO_4) was added and vortexed to ensure the mixing of contents. After the thorough mixing, 200 μL of β-glucuronidase/sulfatase (EC 3.2.1.31) from Helix Pomatia in 0.1 M phosphate buffer was added and vortexed. The mixture was incubated for 1 hour at 37°C to ensure the complete hydrolysis of glucuronide /sulfate conjugates of THC. Then, the THC was extracted with 2 mL of extraction solvent [Ethyl Acetate: Acetonitrile (95:05)] by placing the mixture on a shaker for 10 minutes followed by centrifugation at 4000 rpm at 20°C. The supernatant (organic layer) was transferred into another vial and evaporated to dryness under a stream of nitrogen. The dried residue was reconstituted in 0.3 mL of mobile phase and 20 μL of the reconstituted sample was injected into LC-MS/MS system using auto sampler.

All plasma samples were analyzed on a HPLC system with tandem mass spectrophotometry detection (HPLC/MS/MS) using Merck Zic Hilic, 5 μm, 200°A, 4.6 × 100 mm column and the mobile phase of Methanol: 20 mM Ammonium Formate buffer at the flow rate of 0.8 mL/min. The column oven temperature was set to 50°C during the analysis. Total THC was quantified against standard curve for THC (purity 99%, Sigma-Aldrich).

Plasma concentrations of free curcumin, total curcumin, total DMC, total BDMC and total THC were analyzed using a validated LC/MS/MS method and the following pharmacokinetic parameters were calculated by using "Non-compartmental model" for CURCUGEN and standard curcumin reference product (C-95). Pharmacokinetic parameters evalauted were maximum concentration (C_{max}), area under the curve (AUC_{0-t}), and Time to Maximum Serum Concentration (T_{max}).

Pharmacokinetic parameters (C_{max} and AUC_{0-t}) for total curcuminoids were calculated by using "Non-compartmental model" applied on summation of individual curcuminoids plasma concentrations ie, total curcumin, total DMC and total BDMC. All pharmacokinetic analysis was carried out using Phoenix Version 8.0.

2.6. Safety assessment

The safety assessments included monitoring of AEs, adverse drug reactions, and vital signs monitoring at regular pre-determined intervals and as determined by the medical investigator. Pre-study 12-lead ECG, chest X-ray and urinalysis were conducted for the screening of volunteers. Pre-study hematology and biochemistry assessments were done to select participants with baseline values within reference ranges or clinically non-significant values if outside the reference range. Hematology and biochemistry were repeated in post study to determine any clinically significant abnormality. Breath alcohol test and urine drug screening were done at the time of check-in of each study period to detect participants for any recent substance abuse. A clinical assessment which included general and systemic examination was conducted initially at the pre-study screening. These investigations were carried out for the safety of participants and scientific integrity of the study.

2.7. Statistical analysis

Statistical analysis was performed on pharmacokinetic data of samples assayed and quantified for free curcumin, total curcumin, total DMC, total BDMC and total THC. The analysis was conducted on logarithmically (natural) transformed pharmacokinetic parameters using SAS Enterprise Guide 7.1 version 9.4 for Windows (SAS Institute Inc., Cary, NC). The descriptive statistics (such as N, mean, SD, minimum, maximum, median) were calculated for all the PK parameters for each test and reference products. For T_{max} median was reported as descriptive statistics.

A linear mixed effects model that includes fixed effects terms for Sequence, Treatment, Period and a random effects term for Subject (Sequence) were used. Within the framework of this model and consistent with the 2 one-sided tests for bioavailability, 90% confidence intervals for the difference between test and reference treatment least-squares means for the comparisons Test product vs Reference product was calculated for ln-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of free curcumin, total curcumin, total DMC, total BDMC, and total THC. The differences were considered significant at $P < .05$.

3. Results

Seventeen of the 18 participants planned for the study, were considered for pharmacokinetic and statistical analysis. The

Table 1

Demographic characteristics.

	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m^2)
Mean (SD)	36.6 (4.37)	65.5 (4.58)	165.5 (5.64)	23.9 (1.05)
Median	37.5	65.1	166.3	24.5
Range	26.0–44.0	56.0–71.6	154.5–174.0	22.0–24.9

SD = standard deviation.

demographics of the subjects are presented in Table 1. The sample size of 18 was informed by FDA Center for Drug Evaluation and Research (CDER) Guidance publication for Bioavailability and Bioequivalence Studies (2011, 2014) which recommends a minimum of 12 subjects to satisfy a sufficient power analysis. Whereas no maximum limit is clearly mentioned or required, we planned for 6 additional subjects (n = 18) to power the study even further. Twenty volunteers were admitted for the study, allowing the study to continue sufficiently powered in the event that investigators see fit to remove participants: suffering from inter-current illness, or requiring surgery or the use of unacceptable concomitant medications during the study course, found in violation of the study protocol, having insufficient data points for a meaningful analysis or experiencing any severe adverse effects.

Participants were randomized to the 2 treatment sequences in a random order according to a randomization schedule (using SAS 9.4 or higher version with Enterprise Guide version 7.1) generated by the biostatistician. Study participant numbers were assigned starting from 01 to 18, in the order of the admission to the clinical pharmacology unit. Investigational products were sealed in amber colored containers and placed in self-sealing poly-ethylene sachets by the pharmacist in the presence of a quality assurance person.

Neither the pharmacist or quality assurance person involved in dispensing were involved in the investigational product administration, blood sample collection, evaluation or analysis of AEs. The analyst was blinded towards the identity of the investigational products, and randomization codes were not available to the bio-analytical division until the bioanalytical phase of the study was completed.

Based on 15 samples collected per each participant, over 24 hours from time of administration, the pharmacokinetic parameters of free curcumin, total curcumin, total DMC, total BDMC and total THC, were calculated by using "Non-compartmental model" for the CURCUGEN and Reference product.

All concentrations were plotted using nominal hours post-dose. The mean plasma concentration-time profile of Linear and log-linear graphs for analytes were presented in Figures 1–5. The descriptive statistics (such as N, mean, SD, minimum, maximum, median, %CV) were calculated for all the PK parameters for each test and reference products. For T_{max} median was reported as descriptive statistics. The summary of the Pharmacokinetic Parameter calculations is presented in Table 2.

A linear mixed effects model that includes fixed effects terms for Sequence, Treatment, Period and a random effects term for Subject (Sequence) were used. Within the framework of this model and consistent with the 2 one-sided tests for bioavailability, 90% confidence intervals for the difference between test and reference treatment least-squares means for the comparisons of Test product vs Reference product was calculated for ln-transformed C_{max} and $AUC_{0\text{-}t}$ of free curcumin, total curcumin, total DMC, total BDMC, total curcuminoids and total THC. The differences were considered significant at $P < .05$.

After analysis, the C_{max} and $AUC_{0\text{-}t}$ of the CURCUGEN for free curcumin was 16.1 times and 39 times higher than the C_{max} and $AUC_{0\text{-}t}$ of C-95. The C_{max} and $AUC_{0\text{-}t}$ of CURCUGEN for total curcumin was 25.4 times and 49.5 times higher than the C_{max} and $AUC_{0\text{-}t}$ of C-95. The C_{max} and $AUC_{0\text{-}t}$ of CURCUGEN for DMC was 17.6 times and 43.5 times higher than the C_{max} and $AUC_{0\text{-}t}$ of C-95. The C_{max} and $AUC_{0\text{-}t}$ of CURCUGEN for BDMC was 8.4 times and 46.8 times higher than the C_{max} and $AUC_{0\text{-}t}$ of C-95. The C_{max} and $AUC_{0\text{-}t}$ of CURCUGEN for total curcuminoids was 19.7 times and 52.5 times higher than the C_{max}

Table 2

Pharmacokinetic parameters curcuminoids.

Curcuminoid	Formulation	C_{max} (ng/mL)	AUC_{0-t} (ng h/mL)	T_{max}* (h)	Relative absorption (fold)
Free curcumin	CURCUGEN	1.192^ (1.018)	2.808^ (4.052)	2.00^ (0.50, 8.00)	39.0
	Reference product	0.074 (0.162)	0.072 (0.091)	1.00 (0.00, 2.50)	1.0
Total curcumin	CURCUGEN	193.222^ (51.855)	1225.818^ (694.371)	4.00^ (3.50, 16.00)	49.5
	Reference product	7.621 (2.643)	24.774 (18.214)	2.00 (0.50, 12.00)	1.0
Total DMC	CURCUGEN	125.200^^(34.707	856.944^ (570.432)	4.00^ (3.50, 16.00)	43.5
	Reference product	7.119 (3.726)	19.682 (13.782)	1.50 (0.50, 3.50)	1.0
Total BDMC	CURCUGEN	32.113^ (16.643)	256.946^ (206.725)	3.00^ (1.50, 8.00)	46.8
	Reference product	3.814 (1.696)	5.491 (3.001)	1.50 (0.50, 3.50)	1.0
Total curcuminoids	CURCUGEN	329.399^ (89.369)	2047.485^ (1404.355)	N/A	52.5
	Reference product	16.746 (7.801)	39.001 (33.810)	N/A	1.0
Tetrahydrocurcumin	CURCUGEN	10.948^(4.592)	44.166^ (24.234)	3.00^ (1.50, 6.00)	31.0
	Reference product	0.655 (0.303)	1.427 (0.828)	1.50 (1.00, 3.00)	1.00

The values are given as mean (SD) (n = 17).

* For T_{max}, median (min, max).

^ $P < .05$.

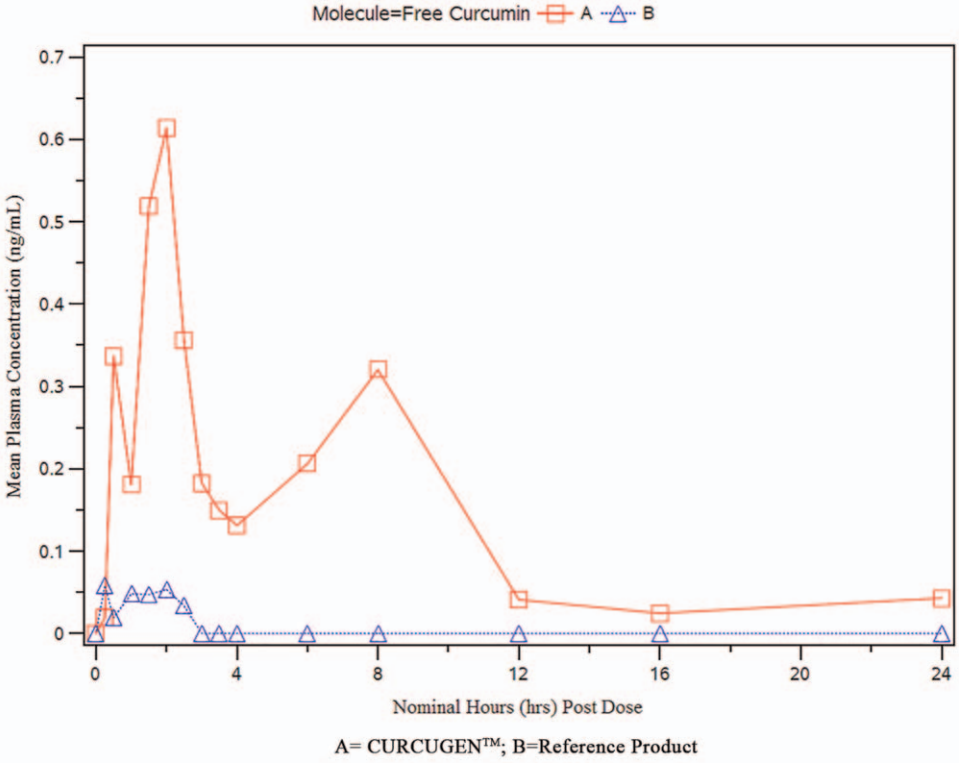

Figure 1. Mean plasma free curcumin concentration vs time plots-linear scale.

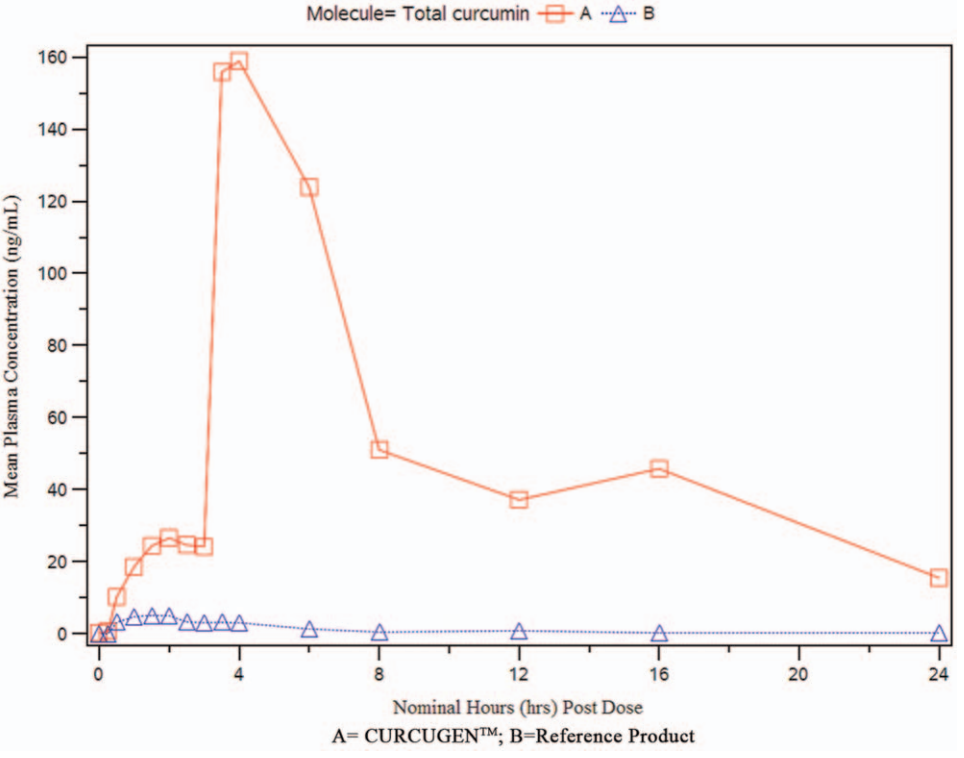

Figure 2. Mean plasma total curcumin concentration vs time plots-linear scale.

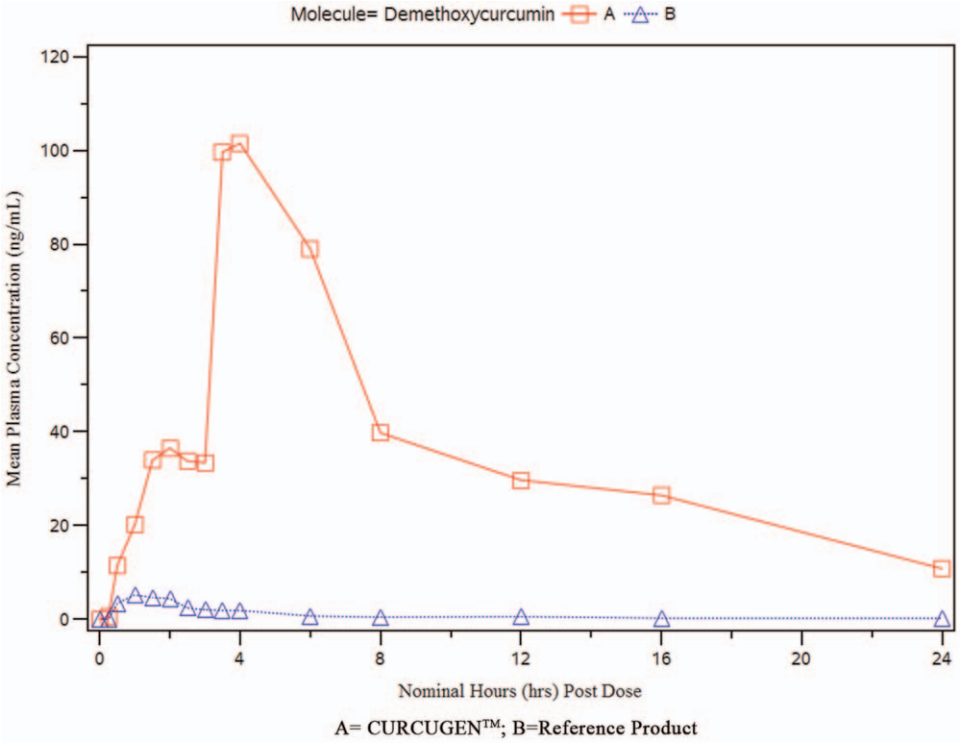

Figure 3. Mean plasma demethoxycurcumin concentration vs time plots-linear scale.

and $AUC_{0\text{-}t}$ of C-95. The C_{max} and $AUC_{0\text{-}t}$ of CURCUGEN for total THC was 16.7 times and 31 times higher than the C_{max} and $AUC_{0\text{-}t}$ for C-95. These results were compiled in Table 2.

The statistical results for the treatment effect on the C_{max} and AUC parameters for free curcumin, demonstrated a statistically significant difference between treatment with CURCUGEN

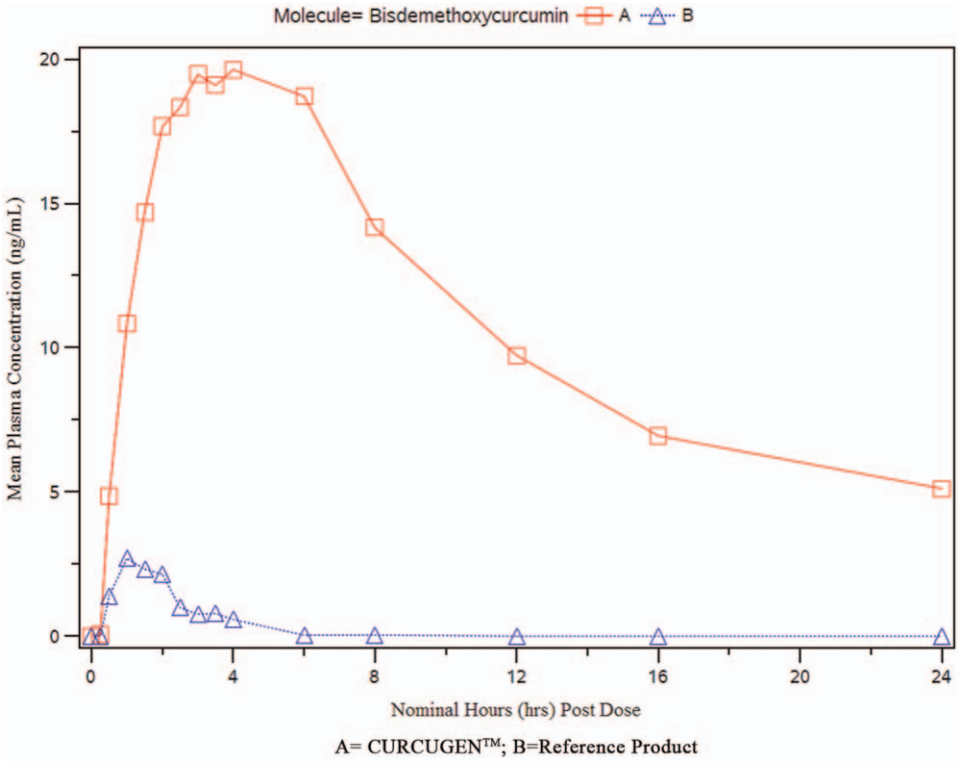

Figure 4. Mean plasma bisdemethoxycurcumin concentration vs time plots-linear scale.

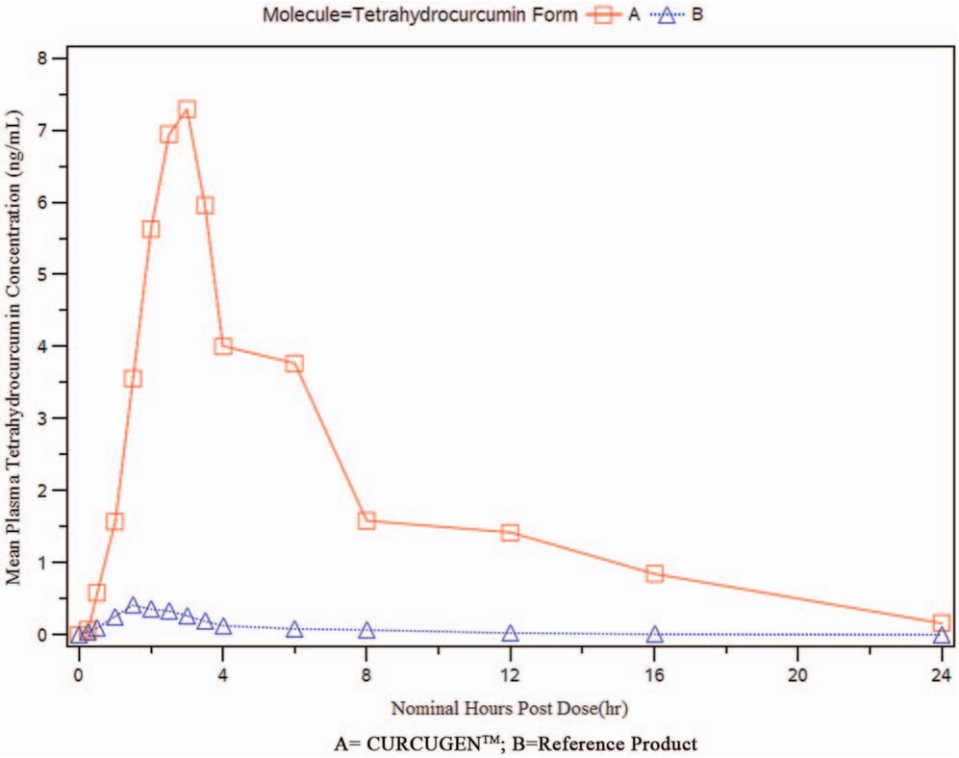

Figure 5. Mean plasma tetrahydrocurcumin concentration vs time plots-linear scale.

versus 95% standardized curcuminoids extract (C-95) at 5% level of significance. It was further evident, from the analysis, that the Sequence Effects and Period Effects on the C_{max} and AUC parameters of free curcumin compared to C-95 were not statistically significantly different at 5% level of significance.

The secondary objective was to establish safety and tolerability of CURCUGEN by monitoring AEs. AE monitoring in the form of clinical examination, vitals check and participant well-being questionnaire were done during the study. There were 2 reported AEs during the study, but were mild in severity and judged to be possibly related the investigational products by study Investigator. Of these 2 AEs, increased eosinophils count occurred in 1 subject that belonged to CURCUGEN group, but was resolved on its own, with no sequelae. The 1 AE of increased ALT occurred in the Reference group, but its resolution was unknown, as the subject was considered lost to follow up. There were no deaths or serious AEs reported during the study.

The completed data analysis from this study supports the primary efficacy measure demonstrating a statistically significant increase in bioavailability of a single oral dose of CURCUGEN over the standard curcumin reference product (C-95) when given under fasting condition. Furthermore, when given under fasting conditions, CURCUGEN appears to have been well tolerated and safe in the study comprising of 18 healthy, adult male human study participants.

4. Discussion

The "best practices" of study design for establishing curcuminoids bioavailability were followed in our study.[14] Potential confounding factors, such as the deliberate or unintentional consumption of turmeric in any form surrounding the dosing and sampling period, were mitigated completely by having the subjects housed and fed within the pharmacology facility from 12 hours pre-dose to 24 hours post-dose. While PK variability was controlled for in the exclusive recruitment of healthy, male participants, our study is limited in its capacity to understand CURCUGEN's precise pharmacokinetics in the female sex. Curcuminoids are differentially metabolized in women versus men. In particular, it has been established that women, due to higher body fat composition and men, due to increased clearance mechanisms can result in pharmacokinetic variances.[29] In order to evaluate pharmacokinetic differences that were independently associated with CURCUGEN unique formulation, this study sought to minimize as many other confounding factors as possible by standardizing the sample population. Otherwise, comprehensive best practice guidelines for the unencumbered evaluation of curcuminoids, included in the noted analysis of curcumin, DMC, BDMC and THC have been applied. To limit confirmation bias, as an example, our study took further precaution in differentially assessing between free curcumin and total curcumin. The delineation of free curcumin versus total curcumin minimizes translational concerns, namely that the assessed bioavailability advantage of a curcuminoid formulation over C-95 is not exaggerated.[27,28,30] Stohs and colleagues in their 2019 study enumerated this, assessing a 31-fold increase in total curcumin after enzymatic hydrolysis.[31]

Total curcumin analysis is an exogenous deconjugation step assessing for the sum of free and (formally) conjugated curcumin. It does not include other curcuminoids. The other curcuminoids, DMC and BDMC were also analyzed in our study using the exogenous deconjugation step to similarly determine total DMC and total BDMC levels, inclusive of their respective free and (formally conjugated) versions. Conjugation of curcuminoids

occurs within intestinal cells or upon first pass through the liver by the adjoining of a water-soluble glucuronide and/or sulfate molecule to the parent compound.[32] Total curcumin analysis applies an ex vivo incubation step in which the plasma sample is incubated with deconjugating enzymes, that is, β-glucuronidase/sulfatase to liberate conjugated-curcumin molecules.[33,34] free curcumin analysis on the other hand evaluates for curcumin absorbed intact into the bloodstream, by assaying for curcumin directly from the plasma sample (i.e., no deconjugation step).[30] Many confounding factors weigh in on the likelihood of an in vitro process of a curcumin-conjugate hydrolysis process translating to what happens in a live system,[31] including the extent of involvement of neutrophils and macrophages – such as with inflammation, pH, and starting concentration of conjugates.[35–37] Whereas curcumin is identified as bioactive, curcumin conjugates as inactive[37] and exogenous curcumin-deconjugation as "variable", we conclude that the differential assessment of free curcumin separate from total curcumin adds further value to current best practice analyses.

Differentially evaluated, CURCUGEN is 39× more bioavailable than C-95 by free curcumin analysis and 49.5× by total curcumin analysis. Analogues of curcumin, DMC and BDMC were evaluated by total DMC and total BDMC analysis resulting in bioavailability enhancements of 43.5× and 46.8×, respectively.

Rounding out the comprehensive pharmacokinetic assessment of curcumin is an appreciation for the larger portion of curcuminoids ingested that does not assimilate directly into the circulation, but that moves along the extent of the gastrointestinal tract. Curcuminoids have been observed to undergo gut bacteria-specific, NADPH-dependent enzyme reduction[37] resulting in metabolites that are structurally more bioavailable, soluble and more bioactive in some regards, than curcumin itself. Many genera and species of bacteria have been identified as biological reactors for curcuminoids, facilitating their transformation into bioavailable, catabolic products.[37] Of these metabolites, THC has received significant attention as a curcumin-derivative whose free radical scavenging capacity outperforms curcumin and whose role appears to provide the missing link between curcumin's well-established gut-brain activity.[4,38,39] Our fidelity to the "best practices" of assessing curcuminoid bioavailability, has established CURCUGEN as the first curcuminoid formulation to evaluate THC pharmacokinetics in the context of total and free curcumin, with an enhanced bioavailability of 31×.

The composite of pharmacokinetic data supporting CURCUGEN as a patent-pending, comprehensively evaluated turmeric extract formulation is posited to be a function of "super-additive assimilation".[14] Super-additive assimilation is described as the benefit consistent with the coupling of diverse delivery strategies, as opposed to a singular mechanism, as customary of currently available formulations. The patent-pending SELF-D, a self-dispersion platform technology used in the production of CURCUGEN facilitates the co-extraction of curcuminoids, essential oils and polar-type resins from the turmeric oleoresin, among other turmeric natives. These additive, non-redundant effects have established CURCUGEN as a highly-bioavailable ingredient with a highly-concentrated turmeric base.

5. Conclusion

CURCUGEN, a novel dispersible, 50% curcuminoids-concentrated turmeric extract significantly increases the bioavailability of free curcumin (39-fold), total curcumin (49.5-fold), total DMC (43.5-fold), total BDMC (46.8-fold), total curcuminoids (52.5-fold) and total THC (31-fold) in comparison to standard curcumin reference product (C-95). CURCUGEN was found to be well tolerated and safe in this study comprising of 18 healthy, adult male human study participants.

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