



Standardisation of Curcuminoids into More Bioavailable form (CurcuminAura™) and Its Pharmacokinetics

**Srikrishna Lingadahalli Puttachar^{a,b*},
Vinisha Mascarenhas^{a,b}, Aliya Mohammad Sayed^{a,b},
Desiree Delicia Fernandes^{a,b}, Teja Moreshwar Godkar^{a,b},
Shawna Reyhan Correia^{a,b}, Saisha Ningappa Patil^{a,b},
Sebastian Thomas^{a,b}, Savitha B Pillai^{a,b},
Sahil Naik^{a,b} and Sakshi Murlidhar Naik^{a,b}**

^a Innovation Centre, DSIR Approved, Department of Research and Development, Verna Industrial Estate, Goa, India.

^b Bio-Med Ingredients Private Limited, Verna, Goa, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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*Corresponding author: E-mail: drkrishna@biomedingredients.com;

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ABSTRACT

Curcuminoids are naturally occurring phytochemicals extracted from the turmeric rhizome *Curcuma longa*, a member of the ginger (Zingiberaceae) family. Through the development of a trademark product called Bio-Curcumin, this study seeks to increase the bioavailability and absorption of regular marketed curcumin by 95%. This finding has important implications for the academic and scientific community. The pharmacokinetic study of CurcuminAura™ and its innovation of biocurcuminoids is detailed in detail. Innovation of BioCurcuminoids and its Pharmacokinetic Study of CurcuminAura™ with regular curcuminoids is significantly described which is the need of the hour. This enhanced sunflower lecithin is an effective ingredient in the trademark product CurcuminAura™, which was created by Bio-Med Ingredients Pvt.Ltd. Its potency in the market can be increased by lecithin's capacity to efficiently encapsulate active substances, improve bioavailability, and increase absorption. Regular curcumin's weak solubility and poor absorption in its free form. Regular Curcumin due to its poor solubility and poor absorption in the free form in the gastrointestinal tract and its rapid biotransformation to inactive metabolites can greatly limit its utility as a health-promoting agent and dietary supplement. Hence to make it more readily available in the body. Curcuminova™ is developed will enhance the properties of Curcumin making it more potent in the market. The Evaluation of the Comparative Pharmacokinetic Study of CurcuminAura™ with Marketed Curcumin 95.0% was carried out through pre-clinical investigations in Sprague Dawley Rats via Oral Route which is the aim of this study with two groups in the study design, with four rats of each sex. Oral administration of CurcuminAura™ and Curcumin 95.0% was administered to rats in the G1 and G3 groups, respectively. A dosage volume of 10 milliliters per kilogram of body weight was maintained for the oral route. After Dosage, under isoflurane, the blood samples were taken from the retroorbital sinuses after the dose was administered under varying periods of anaesthesia. Animals were split up into two sets for each group, and blood samples were taken at 30-, 2-, and 4-hour intervals. Samples were taken for analysis after being kept at -80°C. Designed to increase the bioavailability of curcuminoids, CurcuminAura™ is standardized to 60.9% total curcuminoids by HPLC, as opposed to marked conventional curcumin 95%. This has been confirmed by HPLC analysis. According to comparison studies, CurcuminAura™ has a bioavailability that is 3.8 times greater than the reference standard. Additionally, in this study it is shown that the maximum absorption happens in the timeline 3 hrs after feeding the drug.

Keywords: *CurcuminAura™*; *curcumin*; *zingiberaceae*; *sunflower lecithin*; *pharmacokinetics*; *bioavailability*.

1. INTRODUCTION

Curcuma is one of the bigger genera in the Zingiberaceae family [1]. with roughly 80 species. It is widely distributed over South China, North Australia, Papua New Guinea, Southeast Asia, and tropical India. The herbaceous rhizomatous plant *curcuma* has leaf blades, leafy branches, and underground parts. *Curcuma* is easily distinguishable from other Zingiberaceae genera by its sterile and variously colored coma bracts and blooms borne in cincinni that are subtended by bracteoles and bracts.

Curcuminoids containing curcumin, demethoxycurcumin, bis-demethoxycurcumin, which are extracted and standardised from the rhizomes of *Curcuma longa*. Regular Curcumin has due poor absorption, in the body solubility and poor absorption in the free form in the

gastrointestinal tract and its rapid biotransformation to inactive metabolites can greatly limit its utility as a health-promoting agent and dietary supplement. Hence Comparative Pharmacokinetic Study of CurcuminAura™, with Standard Marketed Curcumin 95% was carried out in Sprague Dawley Rats by Oral Route.

According to the Burt & Smith [2]. 1972 taxonomy, which was long accepted, *Curcuma* was placed in the Hedychieae tribe. The Zingiberaceae family was split up into four tribes using this system. Kress et al. proposed a new system in 2002 [3]. *Curcuma* falls under the tribes category. *Curcuma* was divided into three sections by Baker [4]. Section Hitcheniopsis (characterized by autumnal spikes from the center of the tuft of leaves; bracts are very obtuse, adnate at the sides and spreading at the tip); Section Exantha (the spikes separate from

the shoot); and Section Mesantha (the spikes borne on the shoot either with or without leaves).

Traditional medicine has used turmeric, or *Curcuma longa*, for decades. Turmeric, a spice with a strong flavor that is mostly grown from the roots of a flowering plant found in Southeast Asia and India, is also well-known for its strong anti-inflammatory and antioxidant qualities. Turmeric also lends curry its characteristic bright yellow color.

Curcumin, the main active ingredient in turmeric and the one responsible for the spice's distinctive yellow color, is a member of the Curcuminoids plant compound family. There are several types of turmeric, such as the Lakadong variety from Meghalaya, the Cuddapah variety from Andhra Pradesh, and the Alleppey type from Nizamabad. The HPLC system can identify the other active ingredients, which are DMC - Demethoxycurcumin and BDMC - Bisdemethoxycurcumin as seen in Fig. 1. [5] Curcumin is a naturally occurring antioxidant that possesses anti-inflammatory properties in addition to benefits linked to age reduction, prevention of Alzheimer's disease, and possibly even relief from depression. Turmeric's primary claim to fame is that it is frequently used to reduce inflammation; curcumin is primarily responsible for this effect [6].

Anti-inflammatory substances like curcumin may be useful in the treatment and prevention of a number of cancer types, including colorectal, pancreatic, prostate, breast, and gastric cancers, since inflammation is connected to the growth of tumours. Curcumin may help limit the growth of tumor cells and may even stop tumors from developing in the first place, according to research carried out on mice. Diabetes and its related conditions, such as diabetic nephropathy, also known as diabetic kidney disease, which affects patients with type 1 and type 2 diabetes, may be treated and prevented with curcumin. First of all Turmeric is used as a successful treatment for a number of skin diseases, including acne, eczema (atopic dermatitis), photoaging, and psoriasis because of its anti-inflammatory, antibacterial, and antioxidant qualities. Curcumin is widely utilized in cosmetics and skin care products and has been shown to provide health advantages for kidney and eye health as well as relief from hay fever [7].

The manuscript has been written based on scientific evidence based on the pre-clinical

studies on Sprague Dawley rats. The bioavailability of CurcuminAura™ was compared with regular curcumin to study the curcuminoids. HPLC analysis was carried out and results were analyzed and were represented through chromatographical studies.

1.1 Extraction of Curcumin

The most important stage in recovering curcumin from plant materials is extraction. All extraction techniques were created with a few common goals in mind, including the following: (a) recovering the compounds of interest from plant materials; (b) improving the extraction process' selectivity; (c) increasing extraction efficiency; and (d) offering a reliable and repeatable method [8]. Curcumin is commonly extracted from plants using conventional extraction techniques such as solvent extraction, maceration, and Soxhlet extraction. These techniques are simple but they take a long time, are usually non-selective, and occasionally degrade materials that are sensitive to heat [8]. In order to overcome these challenges, new extraction techniques have been created as more effective substitutes for traditional extraction techniques, such as supercritical liquid extraction.

Traditional techniques for curcumin extraction i.e. Solid-liquid extraction, sometimes called "maceration" or "soaking," is a well-known and frequently employed technique for extracting solid materials from a solvent. Curcumin has been extracted from plants using a wide variety of solvents, such as non-polar organic solvents and a mixture of organic solvents and water [9,10]. In order to isolate curcumin from *Curcuma longa* L., Popuri & Pagala [10] compared the extraction solvents (acetone, ethyl acetone, ethanol, methanol, and isopropanol). It was discovered that when the extraction was carried out at 30°C for 1 hour with a solid to solvent ratio of 1:8, ethanol extraction produced the maximum yield (0.26 mg/10 g). Accordingly, out of all the organic solvents used, ethanol was the most favoured solvent for curcumin extraction [9,11,12]. Furthermore, a significant factor in regulating the extraction efficiency is the solvent mixture's ethanol content [8].

German chemist Soxhlet created the Soxhlet extraction technique in 1879. It is recognized as the gold standard and reference method for the solid-liquid extraction of bioactive chemicals from plants. Several publications have reported on the extraction of curcumin from plants employing a

Soxhlet extractor [13,11]. Another conventional extraction technique used to extract essential oils and bioactive substances is steam or hydro distillation. It has been used to extract the essential oil (turmeric flavor) from raw turmeric powder or oleoresin in order to produce curcumin, or turmeric without flavor. In a study by Silva et al. [14] hydro-distillation produced a good yield of deodorized turmeric at a significantly lower cost than pure curcuminoid pigments. Furthermore, curcuminoids levels in deodorized turmeric were comparable to those in the control and a sample that had undergone hexane extraction for deodorization. These findings suggested that the hydro-distillation process is a productive way to extract curcuminoids or powder with minimal flavor remnants and no loss of pigment.

1.2 Novel Methods for Extraction of Curcumin

1.2.1 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is the most widely utilized non-conventional large-scale industrial technology that uses supercritical fluids as extraction solvents. One of the most widely utilized extraction solvents is supercritical carbon dioxide (CO₂), which is regarded as a green solvent with no toxicity or adverse effects [15,16]. Supercritical CO₂ is efficient because of its thermal and physical characteristics, which combine aspects of its gaseous and liquid forms [17]. When a gas or liquid's temperature and pressure are higher than their critical points, a supercritical fluid can be produced [18]. The SFE process is appropriate for extracting chemicals that are quickly oxidized and thermally unstable

due to its mild processing temperature requirement. Because SFE requires less solvent, extracts data faster, can be automated, and enhances selectivity, it is generally considered a greener extraction technique than classic extraction [15].

1.3 Applications/Uses of Curcumin

Traditional medicine makes considerable use of curcumin. The culinary, cosmetic, and pharmaceutical industries have all seen a rise in demand for it recently. Many research have looked at the uses of curcumin in health, beauty, medical, and cooking products [19-21].

1.4 Curcumin for Medicinal Purposes

Curcumin has a long history of usage in traditional medicine as a home cure and in medicinal applications [22]. Its numerous advantageous biological and pharmacological properties, such as those that are anti-inflammatory, anti-oxidant, anti-bacterial, anti-diabetic, anti-cancer, and anti-tumor, are the main reason for its usage in a variety of medications [19,12,23]. Furthermore, investigations on safety evaluation revealed that curcumin is safe to use at high doses and does not have any harmful side effects [22]. Due to these biological functions that are still active and its extremely safe nature, it has been under more and more scientific scrutiny over the last few decades. Many cancer types, such as bone tumors, brain tumors, head and neck, melanoma, colon, colorectal, pancreatic, breast, prostate, ovarian, lung, and oral cancer, are treated by curcumin [24].

Curcumin:

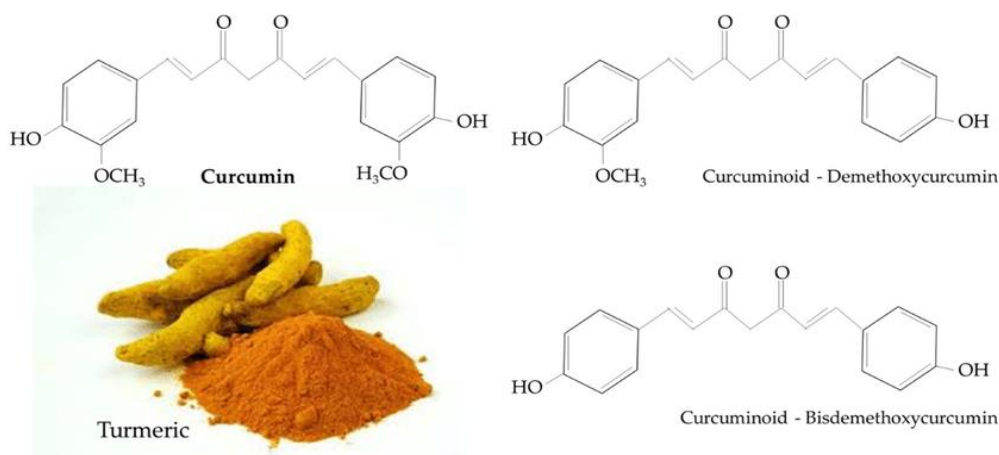


Fig. 1. Structure of curcumin

1.5 Curcumin for Therapeutic Use

Curcumin has a long history of usage in traditional medicine as a home cure and in medicinal applications [19]. Its numerous advantageous biological and pharmacological properties, such as those that are anti-inflammatory, anti-oxidant, anti-bacterial, anti-diabetic, anti-cancer, and anti-tumor, are the main reason for its usage in a variety of medications [19,12,23]. Furthermore, investigations on safety evaluation revealed that curcumin is safe to use at high doses and does not have any harmful side effects [22]. Due to these biological functions that are still active and its extremely safe nature, it has been under more and more scientific scrutiny over the last few decades. Many cancer types, such as those of the bone, brain, head and neck, melanoma, colon, colorectal, pancreatic, breast, prostate, ovarian, lung, and oral regions, are responsive to curcumin's therapeutic activities [25,24]. Furthermore, curcumin has been demonstrated to be a hepato-, nephron-, anti-pathogenic, antiviral, and hypoglycemic molecule [26,24]. Because curcumin possesses anti-inflammatory, anti-infectious, and anti-oxidant properties, it has also been shown to improve wound healing through its role in collagen deposition, granulation tissue development, and tissue remodelling [26].

1.6 Curcumin in the Food Industry

Curcumin, which has a vibrant yellow-orange hue and is a natural food coloring agent that can replace artificial food coloring, has long been used in culinary applications. It improves the appearance of food and is frequently used in rice, beef, mustard, pastries, dairy goods, and canned fish [20]. Moreover, curcumin can extend the shelf life of food by acting as a natural preservative. It is highly effective against a wide range of pathogens, such as *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*. [27]. Research has shown that curcumin effectively preserves cooked mutton, bread, bean curd, and minced meat [28,27]. Since curcumin is extremely sensitive to acid-base reactions, it has also been suggested as a pH-sensitive indicator or sensor for tracking and informing producers, retailers, or consumers about the quality of food. Visual inspection can be used to track color changes in the sensor during food spoiling, and color analysis software can be used to quantify the color changes after canning.

1.7 Curcumin in the Cosmetic Industry

Since curcumin possesses anti-inflammatory and antioxidant properties, the cosmetics industry has been using it for many years. With positive effects against UV light, aging, inflammation, hair loss, lip care, and nail care, it has demonstrated promise for a broad range of cosmetic treatments for the skin, face, hair, lips, and nails. Curcumin has anti-aging, anti-wrinkle, sunscreen, and moisture-retention qualities that make it an effective ingredient in cosmetics [29]. When exposed to a variety of extreme environmental factors, including chemical pollution, UV and infrared radiation, and physical strains, curcumin easily degrades. This helps shield skin from these damaging factors by preventing the production of oxygen free radicals and lipid peroxidation. Notably, formulations containing curcumin have the ability to improve its stability and skin bioavailability (cellular absorption and penetration behavior) [30,21]. Constant curcumin administration improves the aesthetics and self-care of the skin.

1.8 Sunflower Lecithin

Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), and other minor components including carbohydrates and triglycerides make up the majority of lecithins, which are a combination of acetone-insoluble phospholipids [16].

Lecithins are utilized in a variety of commercial applications, including food, cosmetics, pharmaceuticals, dietetics, and more, whether in their natural or modified state [31]. Because of the properties of its phospholipids, this oil industry byproduct serves as a versatile addition for the production of chocolate, instant and baked goods, margarine, and mayonnaise [32]. Lecithin's primary use in the food business is related to its function as an emulsifying agent for emulsions or dispersions. [33]. To make sunflower lecithin, a sunflower must be dried and then divided into three components: solids, gum, and oil [34]. A cold press technique is used to extract the lecithin from the gum. It keeps ingredients from separating and gives meals a smooth, moist texture [13].

1.9 Pharmacokinetics and Bioavailability

The interaction between the characteristics of pharmacodynamics (PDs) and pharmacokinetics

(PKs) determines a molecule's bioavailability (BA). A number of variables, such as PKs (absorption, distribution, metabolism, and excretion; ADME) and PDs, affect how effective a drug is. The presence of xenobiotics, a meal, or a herbal treatment can alter PD responses through various synergistic and negative interactions [35]. By adjusting the ADME and physical characteristics of the active molecule, we can modify the PK and PD properties, and then adjust the BA and bioaccessibility as needed.

As nutraceuticals are complex systems with multiple components and ingredients, their disposition pattern is more intricate than that of a typical synthetic medicine molecule, which is often a single chemical entity. PK models are intended to help comprehend the medicinal moiety's disposition mechanism following absorption. The purpose of the compartment model is to offer a standardized and simple approach to characterize, evaluate, and interpret data obtained from in vivo drug disposal studies. Applications for nutraceuticals, functional foods, and drinks with particular blends of bioactive ingredients have been investigated. Such products can be marketed to certain persons, high-risk patients (with conditions like depression, diabetes, cardiovascular disease, or hypertension), people in particular age groups (such young children, adults, or the elderly), and people with particular dietary needs. In clinical practice, nutraceuticals are typically used alone or in conjunction with other nutrients to target a greater number of biochemical molecules and affect various molecular targets. Considering the variety of active molecules found in supplements and the role that food plays in influencing the

release, dissolution, and ADME (absorption, metabolism, distribution, and excretion) of the active ingredients, it is assumed that nutraceuticals follow multiexponential multicompartment models [36].

2. METHODOLOGY

2.1 Materials

CurcuminAura™ Standardised to 60.9% Curcuminoids, orange to yellow colour powder, belonging to family Zingiberaceae, recommended daily dose is 500 mg, water soluble, shelf life is 3 years at $25 \pm 2^\circ \text{C}$. Marketed Curcumin 95% was used as reference item, Carboxymethyl cellulose (CMC) was used as a vehicle based on solubility trial, 0.1% CMC is universally accepted [37].

2.2 Test System Details

Sprague dawley rats were used for this study, Male and female, nulliparous and non-pregnant having weight 225-295 g for male and 190-248g female. They were obtained from the National Institute of biosciences. Rats support repeat blood collection time points; thus, they are preferred species for evaluation of pharmacokinetic profile [38].

3. METHODS

3.1 Formulation of CurcuminAura™

CurcuminAura™ as seen in Fig. 2; consists of Curcumin 95%, Piperine 95%, Sunflower Lecithin, and Maltodextrin as main ingredients in the formulation.



Fig. 2. Image of CurcuminAura™

3.2 Analytical Comparison of CurcuminAura™ V/S Curcumin 95% By HPLC

CurcuminAura™, was standardized to 60.9% curcuminoids by HPLC, compared to regular marketed Curcumin 95%.

3.3 HPLC Methodology

HPLC Analysis of Curcumin 95% and CurcuminAura™. A Shimadzu P-series HPLC with auto-sampler was operated via Lab solutions software to run the test. The machine includes a UV detector as well as PDA (Photo-Diode array) detector with a 5 µm C-18, shim-pack column of dimensions 4.6 x 250mm. The Isocratic mobile phase consisted of Acetonitrile, double distilled water and ortho-phosphoric acid. The Total run time of the analysis is 25 minutes and wavelength of detection of CurcuminAura™ and Curcumin 95% is 420nm. The Total flow rate is, and injection volume is 20µL. Retention time of BDMC, DMC and Curcumin was observed at RT 15 16 and 18 for Standard and at RT 14, 15 and 17 CurcuminAura™ respectively.

Materials: Acetonitrile and Methanol was of HPLC grade obtained from MERCK; Ortho-phosphoric acid was of Qualigens.

Preparation of Reference standard: Stock solution of Curcumin RS was prepared by dissolving 10 mg in 50 ml methanol.

Preparation of Samples: Stock sample solution of Curcumin 95% and CurcuminAura™ was prepared by dissolving 10 mg in 50 ml methanol.

3.4 Pharmacokinetic Study of CurcuminAura™ V/S Curcumin 95%

3.4.1 Preparation of the dose formulation

Initially formulation trials were conducted to find out the maximum feasible concentration and selection of suitable vehicles. For dose formulation, the required quantity of powder was weighed and triturated using mortar and pestle. A small volume of vehicle was added with continuous stirring to obtain a suspension. The exact quantity of test item and vehicle used was recorded in the raw data. All formulations were prepared fresh, before dose administration. Formulation for the reference item was prepared in a similar manner. The volume of formulation

was prepared based upon the recent animal body weights.

3.5 Experimental Design

3.5.1 Dose administration

Animals from group G1 received a single dose of CurcuminAura™ at 110 mg/kg, respectively by oral route on day 1. Animals from group G3 received a single dose of marketed Curcumin 95.0% at 110 mg/kg, respectively by oral route on day 2. Dose volume was maintained at 10 ml/kg. Actual volume to be administered was calculated based on the recent body weights of each animal.

3.6 Blood Sample Collection and Plasma Separation

As per the number of time points, animals were divided in two sets of two males and females. Post injection blood samples were withdrawn from retro-orbital sinuses, under isoflurane anaesthesia. Sampling time points were 30 mins, 2 hrs and 4 hrs and 1 hr, 3 hrs and 6 hrs. Approximately 0.2 to 0.3 ml blood was collected in vials containing 1% EDTA as an anticoagulant followed by separation of plasma. The separated plasma was transferred to pre-labelled polypropylene tubes and stored at -80°C and then bioanalyzed.

4. RESULTS AND DISCUSSION

4.1 Analytical Comparison of CurcuminAura™ V/S Curcumin 95% By HPLC

CurcuminAura™ is Standardized to 60.9% Curcuminoids content by HPLC, BDMC peak is obtained at RT 14.626, BDMC Content is 1.5%, DMC peak is obtained at RT 15.945, content is 10.89%, Curcumin peak is obtained at RT 17.378, content is 48.6% by HPLC. On comparison to regular Marketed curcumin 95% which has curcumin content 70-80%, DMC 15-20%, BDMC 3-5% by HPLC as seen in Figs. 3, 4, 5 and 6.

4.2 Blood Collection Time Points

The rats were given dose of 110 mg/kg of CurcuminAura™ and regular Curcumin. The blood collection time points by IV route were 30minutes, 1 hour, 2 hours, 3, 4 and 6 hours respectively.

Table 1. Depicting experimental design for CurcuminAura™ v/s (Marketed) Curcumin 95%

Group NO.	Group	Treatment	Dose (Mg/Kg)	Animal Numbers Male	Female
G1	Test Item	CurcuminAura™	110	230501-230504	230505-230508
G3	Reference Item (Marketed)	CURCUMIN 95%	110	230517-230520	230521-230524

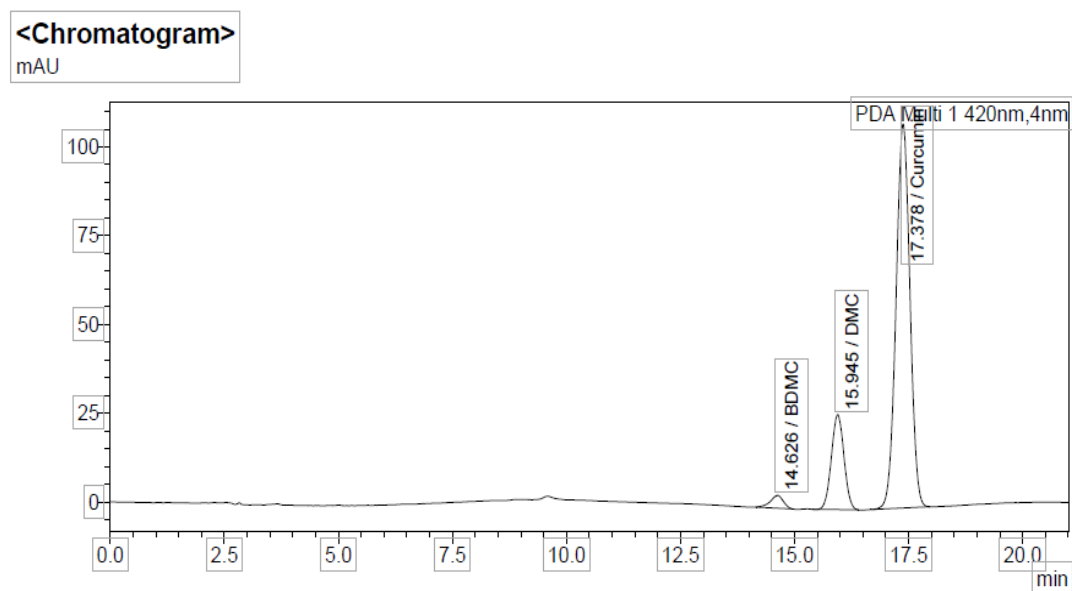


Fig. 3. HPLC chromatogram of CURCUMINAURA™ standardized to 60.9% curcuminoids

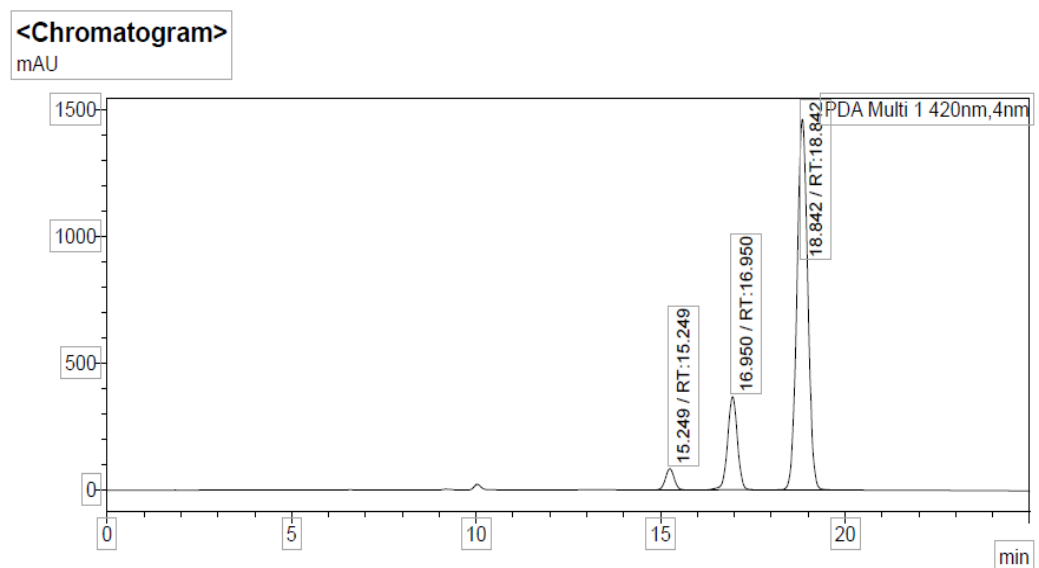


Fig. 4. HPLC Chromatogram of regular marketed curcumin 95%

The above data shows the blood collection time points of CurcuminAura™ v/s Regular marketed Curcumin 95%. Blood is collected at 30minutes, 1 hour, 2, 3, 4 and 6 hours after feeding the test

and reference item at 110mg/kg dose to male/female rats. Maximum absorption is seen after 3 hours of feeding the test item (CurcuminAura™).

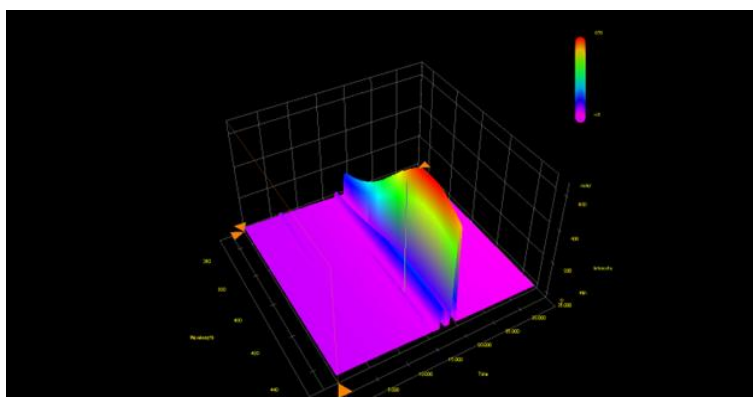


Fig. 5. 3-Dimensional image of curcuminaura™ by HPLC

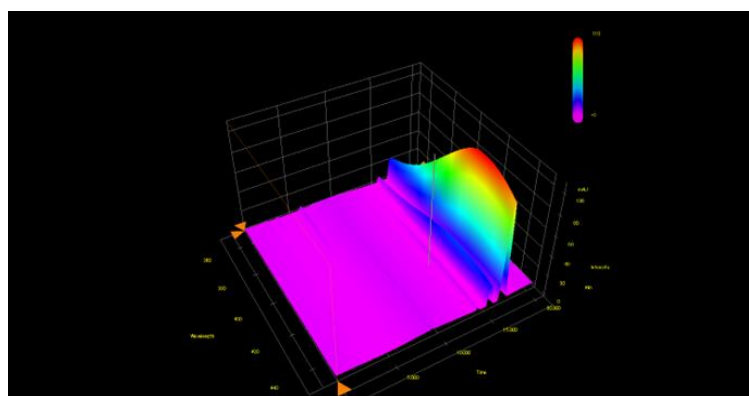


Fig. 6. 3-Dimensional image of regular marked curcumin 95% by HPLC

Blue colour represents CurcuminAura™
 Orange colour represents Curcumin 95%

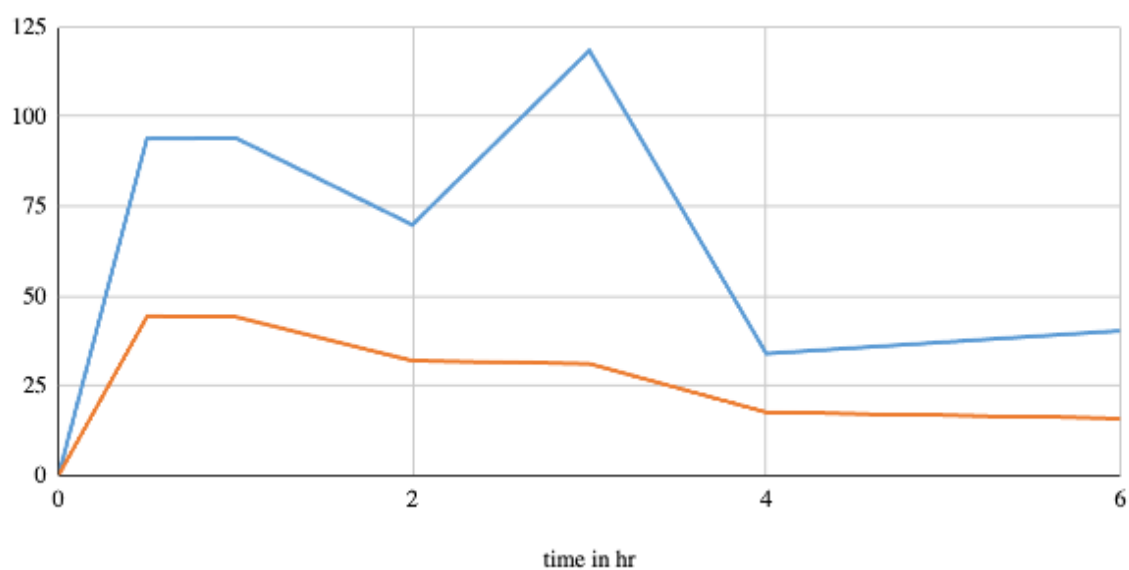


Fig. 7. Graphical representation of curcumin absorption values in ng/ml of curcuminaura™ and curcumin 95% standard

Table 2. Pharmacokinetic parameters of CurcuminAura™

Mean/ SD/N	Blood Time Points			
	0 min	30 min	2hr	4hr
G1 Test Item (CurcuminAura™) Dose: 110 mg/kg				
Mean	0.00	93.96	69.85	33.93
SD	0.00	15.09	7.20	8.52
N	4	4	4	4

Mean/ SD/N	Blood Time Points			
	0 min	1 hr	3hr	6hr
G1 Test Item (CurcuminAura™) Dose: 110 mg/kg				
Mean	0.00	94.10	118.45	40.29
SD	0.00	20.64	112.05	9.71
N	4	4	4	4

Table 3. Pharmacokinetic parameters of regular marketed curcumin 95%

Mean/ SD/N	Blood Time Points			
	0 min	30 min	2hr	4hr
G3 Reference Item (Curcumin 95%) Dose: 110 mg/kg				
Mean	0.00	44.27	31.94	17.58
SD	0.00	8.36	7.08	4.05
N	4	4	4	4

Mean/ SD/N	Blood Time Points			
	0 min	1 hr	3hr	6hr
G3 Reference Item (Curcumin 95%) Dose: 110 mg/kg				
Mean	0.00	44.20	31.11	15.90
SD	0.00	12.29	4.93	6.46
N	4	4	4	4

Table 4. Curcumin absorption values in ng/ml of CurcuminAura™ and curcumin standard

Time in hr	0	0.5	1	2	3	4	6
CurcuminAura™	0	93.96	94.1	69.85	118.45	33.93	40.29
Curcumin 95%	0	44.27	44.2	31.94	31.11	17.58	15.9

5. STUDY OUTCOME AND CONCLUSION

CurcuminAura™ is the turmeric extract standardised to 60% curcuminoids content and formulated to enhance the bioavailability of the curcuminoids. This product has been tested in Rats. The comparison studies evident that CurcuminAura™ has 3.8 times higher bioavailable than the reference standard.

Additionally, in this study it is shown that the maximum absorption happens in the timeline 3 hrs after feeding the drug.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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