



# Report of "Comparative Ex-Vivo Kinetic Study of CURCUIN versus Curcuma longa extract 95% using rat ileum"

Name of Test Product	CURCUIN
Name of Reference Product	Curcuma longa extract 95%
Method to be followed	Kinetic Study Using Rat ileum
Testing Facility	Accuprec Research Labs Pvt. Ltd., Opp. Zydus Pharmez, Changodar – Bavla Highway, Nr. Matoda Patiya, Post – Matoda, Ahmedabad, Gujarat 382213, India.
Sponsor	K. Patel Phyto Extractions Pvt. Ltd. Village Talwada, Behind old sales tax check post, Bhilad, Tal: Umbergaon, Dist. Valsad, Gujarat- 396105, India
Study Period	15 days
Turn around period	30 days
Report Number	ARL/2624/2018
Report Date	08/06/2018

Prepared By (Sign/Date)	Reviewed By (Sign/Date)	Approved By (Sign/Date)
Ms. Kruti Upadhyay	Dr. Manish Rachchh	Dr. Rina Gokani
<b>Research Assistant</b>	(CEO)	(CSO)
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#### I. STATEMENT OF COMPLIANCE

We, the undersigned hereby declare that this Study, entitled "Comparative Ex-Vivo Kinetic Study of CURCUIN versus Curcuma longa extract 95 % using rat ileum" was performed as per standard literature method following OECD principle of GLP. Characterization of the test material was performed by the sponsor. The objective laid down in the study protocol was achieved. No Unforeseen circumstances were observed which might affect the quality or integrity of the study.

The report represents a true and accurate results obtained. We accept the responsibility for validity of the data, as well as the interpretation, analysis, documentation and reporting of the results.

The report comprises of total 13 pages which includes Statement of compliance, quality assurance statement, study personnel detail, experimental design, results, discussion, conclusion, reference and period of archival.

Date: 08 06 2018

106/2018

OCA LISENCE NO. GTUITIN

Ms. Kruti Upadhyay Asst. Study Director

Mr. Samir Patel

Dr. Manish Rachchh Study Director

Dr. Rina Gokani

Q. A. Head







## II. QUALITY ASSURANCE STATEMENT

This study report has been reviewed by the Quality Assurance Unit of Accuprec Research Labs Pvt. Ltd., for compliance with the OECD Principles of GLP

This statement confirms that the study report accurately reflects Study data. The summary of inspections performed during the course of the study is as follows:

Sr. No.	Type of Inspection	Date of Inspection	Phase of Study Inspected
1	Study Based	18/04/2018	Drug Preparation and Dilution
2	Study Based	19/04/2018	Ex-Vivo experiment

Date: 08 06 2018

Mr. Samir Patel 001.612019 SM, QA

Dr. Rina Gokani Q.A. Head







### **1. STUDY INFORMATION**

**Report No.** 

**Study Title** 

Sponsor

**Testing Facility** 

Name of Asst. Study Director Sign of Asst. Study Director

Name of Study Director Sign of Study Director

Name of SM - Q. A. Sign of SM - Q. A.

Name of Q. A. Head Sign of Q. A. Head

: ARL/2624/2018

:"Comparative Ex-Vivo Kinetic Study of CURCUIN versus Curcuma longa extract 95 % using rat ileum".

:K. Patel Phyto Extractions Pvt. Ltd., Village Talwada, Bhilad, N.H.8, Taluka umbezgaon, Dist. Valsad, Gujarat- 396105, India

: Accuprec Research Labs Pvt. Ltd., Opp. Zydus Pharmez, Changodar - Bavla Highway, Nr. Matoda Patiya, Post - Matoda, Ahmedabad, Gujarat 382213, India.

: Ms. Kruti Upadhay Routile 06/2018

: Dr. Man(sh A. Rachchh

: Mr. Samir Patel

:

Sul 05/06/2019

: Dr. Rina Gokani

8/06/2018

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#### 2. SUMMARY

The objective of the study was to perform the Comparative Ex-Vivo Kinetic Study of CURCUIN versus Curcuma longa extract 95 % using rat ileum

## 3. INTRODUCTION

### 3.1. OBJECTIVE

Comparative Ex-Vivo Kinetic Study of CURCUIN versus Curcuma longa extract 95 % using rat ileum

### 3.2. REFERENCE:

Shishu, Manjul Maheshwari. Comparative bioavailability of curcumin, turmeric and Biocurcumax in traditional vehicles using non-everted rat intestinal sac model. *Journal Of Functional Foods* 2010, vol. 2, 60 - 65.

#### 4. STUDY PERSONNEL

Study Director	: Dr. Manish A. Rachchh
Asst. Study Director	: Ms. Kruti Upadhyay
<b>Research Assistant</b>	: Mr. Amol Kharat
Pathologist	: Dr. Prashant Modi
Veterinarian	: Dr. Ashish Patel
DM - Q.A.	: Mr. Samir Patel
Q. A. Head	: Dr. Rina Gokani







### 5. MATERIALS AND METHODS

## 5.1. TEST ARTICLE DETAILS

Test Product Name	Curcuma longa extract 95 %	CURCUIN (Curcuma longa extract 35 %)		
Formulation Code Number	А	В		
Batch No.	KP/CL/044/17	KP/CL/053/17		
Mfg. Date	Dec-2017	March-2018		
Expiry Date	Nov-2020	Feb-2020		
Supplied by	K. Patel Phyto Extractions Pvt. Ltd.,			
	Village Talwada, Bhilad, N.H.8, Taluka umbezgaon,			
	Dist. Valsad, Gujarat- 396105, India			
Stability	The stability of the test article formulations, under the storage			
	conditions used in this study	, was the responsibility of the		
	Sponsor.			
Safety Precautions	Standard laboratory safety	procedure were employed for		
	handling the dose formula	tions. Specifically, Laboratory		
	Apron, Gloves and Face Mask were worn while administer			
	doses.			



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## 5.2 TEST SYSTEM AND ANIMAL HUSBANDRY

Test System	Species: Rat (Rattus norvegicus)		
	Strain: Wistar		
	Sex: Male or Female		
Source	Animal House Facility		
Rationale/Justification for	As per the reference literature rat ileum is preferred		
the Choice of the Test	for Ex-vivo study		
System			
Animal Husbandry			
Test Room	A-103		
Animal House conditions	Lighting : 12 / 12 hour light-dark cycle		
	Temperature: 22 ± 3°C		
	Relative Humidity: 30 to 70%		
	Temperature and relative humidity were recorded		
	Thrice daily.		
Feed and Water	Standard certified rat pellet feed (Manufactured by		
	Keval Sales Corporation, Vadodara) and drinking		
	water treated by reverse osmosis) were provided ad		
	libitum to all animals.		
Sanitation	The floor of the experimental room was swept and		
	mopped thrice daily. Cages and bedding materia		
	was changed once in three days and water bottles		
	were changed daily. All the experimental		
	procedures to be done in a clean environment.		







#### 6 EXPERIMENTAL DESIGN

#### 6.1 SOURCE OF TEST ITEM:

Test Drug, Curcuma longa extract 95 % (Formulation A) and CURCUIN (Formulation B) was provided by the sponsor.

### 6.2 SOLUBILITY STUDY IN WATER AND METHANOL

Solubility of CURCUIN and Curcuma longa extract 95 % was determined using water and methanol as solvent 25 mg of CURCUIN was added to 50 ml of Distilled Water and methanol. In two separate beaker respectively. Similarly 25 mg of curcuma longa extract 95 % was added to 50 ml of Distilled Water and methanol in two different separate beaker. And kept at 37 ± 1 °C in a thermo-stated shaking water bath for 10 min. Then contents were then filtered through 0.22 µm filter. Different dilution of filtrate were prepared using krebs ringer phosphate buffer saline with IPA Solution (7:3). The samples were analyzed at  $\lambda_{max}$  422 nm using a UV–visible spectrophotometer.

### 6.3 DRUG PREPARATION & STANDARD CURVE PREPARATION

The solution of curcuma longa extract 95 % and CURCUIN were prepared by weighing 50 mg of curcuma longa extract 95 % and CURCUIN and added to 100 ml of Distilled Water in Conical Flask and was kept at  $37 \pm 1$  °C in a thermo-stated shaking water bath for 24 h. Then contents were then filtered through 0.22 µm filter. Different dilution of filtrate were prepared using krebs ringer phosphate buffer saline with IPA Solution (7:3) and absorbance was measured at at  $\lambda$ max 422 nm using a UV–visible spectrophotometer.

### 6.4 PREPARATION OF KREBS RINGER PHOSPHATE BUFFER SALINE

It is prepared using sodium chloride (0.67%, w/v), potassium chloride (0.034%, w/v), magnesium sulphate (0.059%, w/v), calcium chloride (0.011%, w/v), sodium dihydrogen phosphate (0.234%, w/v) and glucose (0.18%, w/v) in distilled water and isopropyl alcohol in the ratio of 7:3 (v/v)

#### 6.5 EXPERIMENTAL PROCEDURE FOR EX-VIVO STUDY:

In study, male Wistar rats (weighing 200–250 g) were used. Animals were sacrificed by spinal dislocation after overnight fasting. The small intestine was removed by cutting across the upper end of the duodenum and the lower end of the ileum. and





manually stripping the mesentery. The small intestine was washed out carefully with cold normal oxygenated saline solution (0.9%, w/v, NaCl) using a syringe equipped with blunt end. The clean intestinal tract was prepared into 8 ± 0.2 cm long sacs having a diameter of  $3.0 \pm 0.5$  mm. Each sac (A1-A3 and B1-B3) was filled with 1 ml of curcumin A and B formulation (equivalent to 0.5 mg of curcumin) via a blunt needle, and the two sides of the intestine were tied tightly with thread. Each non-everted intestinal sac was placed in a glass conical flask containing 50 ml of a mixture of Krebs Ringer phosphate buffer saline pH 7.4 and isopropyl alcohol in the ratio of 7:3 (v/v). The entire system was maintained at 37 °C in a shaking water bath operating at 50 rpm and aerated with oxygen using laboratory aerator. From outside of the sac 4 ml samples were withdrawn at time 1 h, 3 h, 5h, 7h and replaced with fresh 4 ml of medium. The samples were analyzed at  $\lambda_{max}$  422 nm using a UV–visible spectrophotometer.

Values of concentration for test sample were derived from equation received though standard curve as mentioned above in 6.3

#### 6.5 **OBSERVATIONS**:

Absorbance was measured at  $\lambda$ max 422 nm using a UV-visible spectrophotometer. In above mentioned various study objectives, standard curve and graph was prepared.

In case of penetration study, the concentration of formulation was derived from absorbance recorded and back calculated using standard curve obtained from respective formulation study.

### 6.7 STATISTICAL ANALYSIS:

All data were represented as Mean.







7 RESULT

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- 7.1 The Solubility of CURCUIN and curcuma longa extract 95 % data were attached by Annexure I
- 7.2 The Standard Preparation curve of CURCUIN and curcuma longa extract 95 % were attached by **Annexure II**
- 7.3 Permeability Study of CURCUIN and curcuma longa extract 95 % at 1 hr, 3 hr, 5 hr and 7 hr data attached by **Annexure III**

### DISCUSSION AND CONCLUSION

- 8.1 From the solubility study result, it can be concluded that CURCUIN possess high solubility in water after 10 min of mixing, whereas curcuma longa extract 95 % was practically insoluble in water after 10 min.
- 8.2 From the Ex-vivo Kinetic study data, it can be concluded that CURCUIN possess significantly high bio-absorption as compared to curcuma longa extract 95 % after 7 h.

Dr. Manish Rachchh Study Director



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#### 9 ARCHIVES

All original raw data, draft report and final report will be retained in the archives of Accuprec Research Labs Pvt. Ltd., Ahmedabad, Gujarat, India for a period of 9 years. At the end of the archiving period, the Sponsor's instructions will be sought either to extend the archiving period or to return or dispose of the archived material.

Date: 08/06/2018 Row # 106/2018

Ms. Kruti Upadhyay Asst. Study Director

Mr. Samir Patel SM, QA

Yee path

Dr. Manish Rachchh Study Director

Dr. Rina Gokani Q. A. Head





### **III. CERTIFICATE**

This is to certify that the test material CURCUIN and Curcuma longa extract 95 %, supplied by K. Patel Phyto Extractions Pvt. Ltd. Village Talwada, Behind old sales tax check post, Bhilad, Tal: Umbergaon, Dist. Valsad, Gujarat- 396105, India for comparative Ex-Vivo Kinetic Study of CURCUIN versus Curcuma longa extract 95 % using rat ileum.

The test formulation CURCUIN (Curcuma longa extract 35%) possessed significantly high bio-absorption as compared to curcuma longa extract 95 % after 7 h study in the present Ex-vivo study using rat ileum.

Accuprec Research Labs Pvt. Ltd., is approved for experiment on laboratory animal by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

Accuprec Research Labs Pvt. Ltd., is approved by the Food & Drug Administration, Gujarat State, Gandhinagar, through License No. GTL/37/31.

Date: 08 06 20 18 3/06/2018 Ms. Kruti Upadhyay Asst. Study Director

Ultimate solution ...

Dr. Manish Řačhchh Study Director

Dr. Rina Gokani Q. A. Head





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Annexure I

#### Solubility study of CURCUIN (Curcuma longa extract 35 %) & Curcuma longa extract 95 %

**Study Title:** Comparative In-Vitro Solubility Study of Curcuma longa extract 35 % versus Curcuma longa extract 95 % using methanol as standard solubilizer

CURCUIN (Curcuma longa extract 35 %)			
Concentration (ug/ml)	Distilled Water	Methanol	
	Absorbance	Absorbance	
25	0.026	1.42	
50	0.056	2.553	
100	0.109	3.425	
150	0.147	3.599	
200	0.179	3.644	
250	0.188	3.6996	

Curcuma longa extract 95 %

**Distilled Water** 

Methanol



	Absorbance	Absorbance
25	0.001	3.308
50	0.001	3.434
100	0.001	3.708
150	0.002	3.912
200	0.002	3.913
250	0.002	4

#### **Result Discussion:**

Concentration

(ug/ml)

AS per the data displayed in table and dipiected in graph, considering Methanol as a standard solven in which Curcumin is highly soluble, while comparing both Curcuma longa extract 95% and Curcuin, study suggested that Curcuma longa extract 95% is practically insoluble and sparingly dispersable in water whereas Curcuin is approximately 99 times more dispersable.



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#### **Result Discussion:**

Data displayed in table and shown in graph suggested that Curcuin was almost 20 times more dispersable than Curcuma longa standard extract 95% in Krebs ringer solution.



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Annexure III

#### Permeability Study

Study Title: Comparative Ex-Vivo Kinetic Study of CURCUIN (Curcuma longa extract 35%) versus Curcuma longa extract 95 % using rat ileum

Curcuma longa extract 95% (Formulation A)					
Absorbance					
Time (Hr) A1 A2 A3 Aver					
1	0.017	0.016	0.019	0.017	
3	0.018	0.017	0.02	0.018	
5	0.02	0.019	0.022	0.020	
7	0.022	0.021	0.024	0.022	

CURCUIN (Curcuma longa extract 35%) (Formulation B)					
	Absorbance				
Time (Hr) B1 B2 B3 Averag					
1	0.024	0.028	0.026	0.026	
3	0.026	0.034	0.042	0.034	
5	0.049	0.051	0.053	0.051	
7	0.052	0.054	0.056	0.054	

Time (Hr)		Concentrat	tion (ug/ml)	
rine (rir)	A1	A2	A3	Mean
1	4	2	8	4.67
3	6	4	10	6.67
5	10	8	14	10.67
7	14	12	18	14.67

Time (Hr)	Concentration (ug/ml)			
	B1	B2	B3	Mean
1	40.5	60.5	50.5	50.50
3	50.5	90.5	130.5	90.50
5	165.5	175.5	185.5	175.50
7	180.5	190.5	200.5	190.50





#### **Result Discussion:**

Data displayed in table and depicted in graph suggested that permiability of Curcuin through rat ileum was consistently increasing up to 7 hrs and was almost 13 times more than Curcuma longa standard extract 95% at the concentration of 500ug/ml after 7 hrs. Considering the fact that Curcuin is containing 35% curcumin it can be postulated that Curcuin is approximately 39 times more bio-available than standard Curcuma long extract 95%.

Due to very low injectable volume in rat ileum and consequently very low permiability of standard drug i.e. Curcuma longa extract 95%, assessable concentration in Krebs Ringer solution was found very less and not detectable for HPLC analysis. Hence both the samples were quantified by UV spectrophotometry. Present study proved that Curcuin was almost 39 times more permiable through rat ileum than Curcuma longa standard extract 95%. While corelating water solubility data with the results of these experimental model, it is assumed that in better experimental condition, bioavailability of Curcuin may be found more.



08/06/2018