Indo Global Journal of Pharmaceutical Sciences, 2013; 3(1): 40-51

INDO GLOBAL JOURNAL OF PHARMACEUTICAL SCIENCES
ISSN 2249- 1023

# Formulation & Development of Anti-Obesity Liquid Formulation Containing *Garcinia Cambogia* Extract, L-Carnitine & Chromium Picolinate

Rakesh Patel[a], Jaimin Patel[b], Saloni Kakkar[c*]

[a] *Indubhai Patel College of Pharmacy & Research Centre(IPCPRC), Dharmaj, Gujarat, India*

[b] *Roaq Chemicals Pvt. Ltd., Baroda, India*

[c] *Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, India*

**Address for Correspondance**: Saloni Kakkar , salonikakkar2007@gmail.com

**ABSTRACT:** The aim of the current investigation is to develop antiobesity liquid formulation of Garcinia cambogia extract, L-Carnitine and Chromium Picolinate, which gives good microbial stability and pharmacological activity. The formulation was prepared by using coconut water as base and flavour in different combinations. The prepared formulation was evaluated for general description, microbiological and drug content. The formulation showed acceptable physical, microbiological properties and complied with drug content limits. The results of assay of Garcinia cambogia (HCA content) was 108.23% and 108.16%, before and after stability study, by HPLC. L-Carnitine content in formulation was 106.15% and 105.58%, before and after stability study, by HPLC. Chromium picolinate content was 99.2% and 97.8%, before and after stability study, by AAS. From the results it was concluded that the formulation was relative stable. 

**KEYWORDS:** *Garcinia Cambogia* Extract; L-Carnitine; Chromium Picolinate; HPLC; AAS.

## INTRODUCTION

One of the most serious and the fastest growing public health problems throughout the industrialized world is Obesity. With visceral fat accumulation obesity is a serious risk factor for so-called metabolic syndrome, which includes insulin resistance, glucose intolerance, hypertension, and dyslipidemia[1]. A person is considered to be obese if the bodyweight is 20% higher than it should be. BMI between 25 and 29.9 is considered as overweight. If your BMI is 30 or over you are considered obese. Abnormal or excessive fat accumulation that may impair health is considered as overweight and obese.

Simple index of weight-for-height that is commonly used to classify overweight and obesity in adults is known as Body Mass Index (BMI). This can be defined as a person's weight in kilograms divided by the square of his height in meters $(kg/m^2)^2$.

The aim of the present research is to investigate the anti-obesity effect of a mixture composed of *Garcinia cambogia* extract, chromium picolinate, and L-Carnitine (1.2:0.3:0.02, w/w/w) in obese people to promote weight-loss and lower plasma cholesterol. Broadly, the work would endeavor to achieve the following objectives:



eISSN: 2249 1023

- To prepare an oral liquid formulation containing L-Carnitine, Garcinia cambogia extract and chromium picolinate for the treatment of obesity.
- To study the Preformulation factors such as saturation solubility of drugs, drug-excipients interaction, analysis of drugs, etc.
- To characterize prepared liquid for Physical appearance, Assay, Stability, Microbiological tests.

**Rationale for selection of drugs:**

The oral liquid preparation contains 3 APIs: L-carnitine, Garcinia cambogia extract (Hydroxy citric acid) and Chromium picolinate. All the drugs have potential effect on obesity. These drugs have different mechanism on obesity hence have synergistic effects.

**L-Carnitine**: Carnitine transports long-chain acyl groups from fatty acids into the mitochondrial matrix, so they can be broken down through β-oxidation to Acetyl CoA to obtain usable energy via the citric acid cycle.

**Garcinia cambogia extract (Hydroxy citric acid):** Garcinia Cambogia fills the glycogen stores in the liver and other tissues, thereby reducing appetite while increasing energy levels. Garcinia Cambogia lowers the production of triglycerides and cholesterol and may also increase thermogenesis, the burning of calories.

**Chromium picolinate:** Chromium picolinate works by stimulating the activity of insulin, thus significantly aiding the body's glucose and fat metabolism, managing the breakdown of glucose and fat.

**Rational for selection of dosage form:**

Oral Liquids are homogeneous liquid preparations, usually consisting of a solution, an emulsion or a suspension of one or more medicaments in a suitable vehicle. In this formulation, all drugs; L-carnitine, Garcinia cambogia and Chromium picolinate; are easily soluble in water (coconut water) and form a solution.

The dose of these drugs is higher: L-carnitine: 2-6 gm/day, Garcinia cambogia: 0.75- 3.0 gm/day and chromium picolinate 0.5-1.5 gm/day. So this cannot be formulated as single tablet form, but can easily be formulated as liquid form. Also oral liquids can be easily taken by the all patients (children, geriatrics)

## MATERIALS & METHODS

The following manufacturing formula was considered for the initial trials.

**General procedure of Formulation:**

Accurately weighed Garcinia cambogia extract, L-Carnitine and chromium picolinate and were dissolved in water in a 100 ml volumetric flask. Then the antimicrobial agents, methyl paraben and propyl paraben were added in the concentration range of 0.05, 0.1, 0.15, and 0.2%. Among all concentrations 0.2% of methyl paraben and propyl paraben was found to be optimum. Then coconut water was added in the concentration range of 20, 30, 50, 70, and 90ml in 100ml of formulation. Among this 90ml of coconut water was found to be optimum. And finally the volume was made up to 100ml with water. Different formulations were prepared and evaluated. Among this the formula as shown in table 2 was found to be optimum after the evaluation of different formulas.

**EVALUATION OF FORMULATION:**

**A. General appearance:** [3, 4]

The general appearance is a visual identity, essential for consumer acceptance to confirm lot to lot uniformity and for monitoring trouble free manufacturing. The control of the general appearance of solution involves the measurement of colour, odour, taste etc.

**B. Bulk density ($D_o$)**[5]

It is the ratio of bulk volume to the total mass of the powder taken. It can be measured by pouring the weighed powder into a graduated cylinder and the volume was noted. It is given by:

$$D_o = M/V_o$$

Where 'M' is the mass of powder,



eISSN: 2249 1023

'$V_o$' is the Bulk Volume of powder; it is expressed in gm/ml.

| Sr. No | Ingredients | Category |
|---|---|---|
| 1. | Garcinia cambogia extract | Active Pharmaceutical Ingredient |
| 2. | L-Carnitine | Active Pharmaceutical Ingredient |
| 3. | Chromium Picolinate | Active Pharmaceutical Ingredient |
| 4. | Methyl paraben and Propyl paraben | Antimicrobial preservatives |
| 5. | Coconut water | Flavor base |
| 6. | Distilled water | Solvent |

**Table 1 Proposed manufacturing formula**

| Sr. No | Ingredients | Quantity taken |
|---|---|---|
| 1. | Garcinia cambogia extract | 3gm |
| 2. | L-Carnitine | 500mg |
| 3. | Chromium Picolinate | 16mg |
| 4. | Methyl paraben | 0.2% |
| 5. | Propyl paraben | 0.2% |
| 6. | Water | 10ml |
| 7. | Coconut water | Upto 100ml |

**Table 2 Optimized formula**

**A. Tapped density (Dt)** [5]

It is the ratio of mass of the powder to the tapped volume of the powder. The tapped volume was measured by bulk density apparatus in which the powders were tapped for predetermined number of taps until the volume remained constant. It is given by:

$$D_t = M/V_t$$

**B. Determination of moisture contents**[6,7]

**Materials required:** Evaporating dish, Hot plate, Desiccators, Weighing balance, Sample powder

**Procedure:** About 100 gm accurately weighed drug (without preliminary drying) was placed in a tarred evaporating dish, and dried at 105°C for 1 hour in oven. Continue the drying and weighing at an interval of 30 min until difference between two successive weighing corresponds not more than 0.25%. Constant weigh is reached when two consecutive weighing after drying for 50 minutes. Cool for 30 minutes in desiccators, show not more than 0-1 gm difference.

**C. Determination of pH value:** [3,4]

The pH value of a solution can be determined potentiometrically by using glass electrode as a reference electrode and a digital pH meter. The apparatus was properly calibrated, the electrode was immersed in the solution and the pH was measured.

**D. Determination of specific gravity:** [3,4]

It is defined as the ratio of the density of a substance to the density of the water, the value for both substances being determined at the same temperature unless otherwise specified. It is determined by using pycnometer. The formulation was filled up to the mark in pycnometer and weighed and same way water was filled up to the mark and weighed in same pycnometer. The specific gravity was determined by the following formula:

**Specific gravity = Weight of substance/Weight of an equal volume of water**

**E. Determination of relative viscosity:** [3,4]

Viscosity of any liquid is measured by comparing it with the viscosity of water. It was measured by Oswald Viscometer.



eISSN: 2249 1023

Water was filled up in Oswald Viscometer and sucked it up to the mark then allowed it to run from mark A to B and time required for this run was noted. The same procedure was followed for formulation.. Viscosity of the formulation was measured by the following formula:

$$\eta_1/\eta_2 = d_1t_1/d_2t_2$$

where $\eta_1$ and $\eta_2$ are the viscosity of liquid and water respectively while $t_1$ and $t_2$ are time taken for reaching water and liquid from mark A to B in seconds and $d_1$ and $d_2$ are the density of liquid and water respectively.

**F. Microbiological test:** [3,4]

10 ml of sample was accurately measured and transferred to 90 ml of sterile phosphate buffered working solution flask. Content was mixed well and 1 ml of above solution was transferred in to two set of sterile petri plates. About 20 ml sterile soyabean casein digest agar media (previously cooled at 40°c) was aseptically transferred. Mixed well, allowed to solidify. The plates were transferred to an incubator at temperature of 37+ 2°C for a period of 24 hours. On second day the plates were examined for growth of microbes and the colony forming units (CFU) was counted by using electronic colony counter. Same procedure was followed for up to 7 days. The growth of microbes was noted.

**For yeast and moulds:**

Same procedure was followed as above using Sabouraud dextrose agar in place of soya bean casein digest agar media. The growth of yeast and moulds were noted.

**A. Assay of APIs**

**Garcinia cambogia: assay by HPLC**

**Assay procedure:**

The HPLC chromatograms for the standard and sample solution were recorded using the set chromatographic conditions. The analytical run time was 10 min and the retention time for HCA was 2.5 + 0.5 min.

| Chromatographic conditions: |
|---|
| Pump system: Shimadzu LC-20AT |
| Injector: Shimadzu SIL-HTc Auto Sampler |
| Detector: Shimadzu SPD-M20A PDA detector |
| Auto sampler: Shimadzu SIL-HTc Auto Sampler |
| Column: Phenomenex Luna C18 (2), 5µm, 4.6x150mm |
| Filtering system: Advance Micro devices pvt.ltd. PTFE 0.45 µm |
| Wavelength: 210 nm |
| Injection volume: 20 µl |
| Flow rate: 1.5 ml/min |
| Mobile phase: A: 0.2% phosphoric acid |
| B: Acetonitrile |

**Table 3 Chromatographic conditions for the assay of Garcinia Cambogia Extract**

| After min > | 00 | 05 | 10 | 10 |
|---|---|---|---|---|
| **MP A** | 100 | 00 | 10 | Stop |
| **MP B** | 00 | 00 | 90 | Stop |

**Table 4 Mode of separation: Gradient**



eISSN: 2249 1023

**Calculations:**

Amount of active present % = Area of sample x conc. of standard x purity of standard / Area of standard x conc. of sample

**L-Carnitine: Assay by HPLC** [5,8,9]

**Materials:**

Ammonium acetate, Acetonitrile, Water, Acetic acid, L-Carnitine USP reference standard

**Assay procedure:**

The HPLC chromatograms for the standard and sample solution were recorded using the set chromatographic conditions.

**Table 5: Chromatographic conditions for the assay of L-Carnitine**

| Chromatographic conditions: |
|---|
| Pump system: Shimadzu LC-20AT |
| Injector: Shimadzu SIL-HTc Auto Sampler |
| Detector: Shimadzu SPD-M20A PDA detector |
| Auto sampler: Shimadzu SIL-HTc Auto Sampler |
| Column: Phenomenex Luna C18 (2), 5µm, 4.6x150mm |
| Filtering system: Advance Micro devices Pvt.ltd. PTFE 0.45 µm |
| Wavelength: 286 nm |
| Injection volume: 20 µl |
| Flow rate: 1.0 ml/min |
| Mobile phase: A: 0.1 M Ammonium acetate (90) |
| B: Acetonitrile (10) |

**Calculations:**

Amount of active present % = Area of sample x conc. of standard x purity of standard / Area of standard x conc. of sample

**Chromium picolinate: Assay by AAS (USP)** [5]

**Procedure:**

The absorbance of the standard preparation and the sample preparation were concomitantly determined at the chromium emission wavelength of 357.9 nm, with a suitable atomic absorption spectrometer equipped with a chromium hollow cathode lamp and an air acetylene flame, using dilute nitric acid as the blank.

Calculated the quantity in mg of $C_{18}H_{12}N_3O_6Cr$ in the portion of chromium picolinate taken by the formula:

**(418.31/51.996) x (10 C)**

Where in C is conc. in µg/ml of chromium in the assay preparation

418.31 = Molecular Weight of chromium picolinate

51.996 = atomic weight of chromium.

**Stability study** [3,4]

The ICH Guidelines have established that long term stability testing should be done at 25°C/60% RH; Accelerated stability testing should be done at 40°C/75% RH for 6 months. If significant change occurs at this condition, then the formulation should be tested at an intermediate condition 30°C/75% RH.



eISSN: 2249 1023

In the present work stability study (accelerated) was carried out for the optimized formulation to check it's stability by using following condition and time period: 25°C+2°C/60%RH+5%RH because the storage condition of formulation is below 18°C.

## RESULTS & DISCUSSION

**Preformulation study of materials:**

Preformulation study of the materials is important for the optimization of different parameters of formulation. Physical parameters of the material are important in the identification of the materials. Particle size is the important parameter for the flow properties of the material whereas bulk density and tapped density of the material is of concern for flow characteristics of the material.

For Garcinia cambogia extract, bulk density and tapped density limit is 0.4-0.8 gm/ml and 0.5-0.9 gm/ml respectively, for the good flow characteristics. The result for bulk density and tapped density of extract was found to be 0.7 gm/ml and 0.85 gm/ml respectively; hence it had good flow properties.

Moisture content is an important parameter for the storage and stability of the material. If the material is hygroscopic, then it should be stored in a dry place. Some materials can degrade in the high moisture condition. Both Garcinia cambogia extract and L-Carnitine are hygroscopic material, so it should be stored in a dry place. Solubility of the material is most important parameter for any formulation. Garcinia cambogia extract and L-Carnitine, both are highly soluble in water. So it is easy to prepare the liquid formulation of both these ingredients. Medicinal plants may be associated with a broad variety of microbial contaminants, like bacteria, moulds etc. This microbiological background depends on several environmental factors, methods of harvesting, cleaning, drying, handling, and storage. It exerts an important impact on the overall quality of herbal product and preparations. For the Garcinia cambogia extract TPC and yeast-moulds limit is <10000 cfu/gm and <200 cfu/gm respectively. Results obtained were in the limit, so the quality of formulation is good.

**Evaluation of formulation :**( Optimized formula)

| Sr no | Parameter | Garcinia Cambogia extract | L-Carnitine |
|---|---|---|---|
| 1. | Appearance | Granular powder | Crystalline powder |
| 2. | Colour | Off white to gray | White |
| 3. | Odour | Characteristic | Characteristic |
| 4. | Taste | Sour and chalky | Salty |
| 5. | Particle size | 6.80% on 60# | 17% on 20# |
| 6. | Bulk density | 0.70 g/ml | 0.66 g/ml |
| 7. | Tapped density | 0.85 g/ml | 0.77 g/ml |
| 8. | Moisture contents | 2.65% | 1.85% |
| 9. | Solubility | Freely soluble in water | Freely soluble in water |
| 10. | Micro : TPC<br>Yeast and Moulds | 2500 cfu/gm<br>70 cfu/gm | -- |

**Table 7 Results of Preformulation study**

| Sr No | Parameter | Result |
|---|---|---|
| 1. | Appearance | Turbid solution |
| 2. | Colour | Brown |
| 3. | Odour | Characteristic |
| 4. | Taste | Salty |
| 5. | pH | 4.95 |
| 6. | Specific gravity | 1.28gm/ml |
| 7. | Viscosity | 24.6 poise |

**Table 8: Results of Q.C. parameters of formulation**



eISSN: 2249 1023

**Results of Assay of APIs:**

**Garcinia cambogia (HCA content): by HPLC**

**A. Before stability study:**

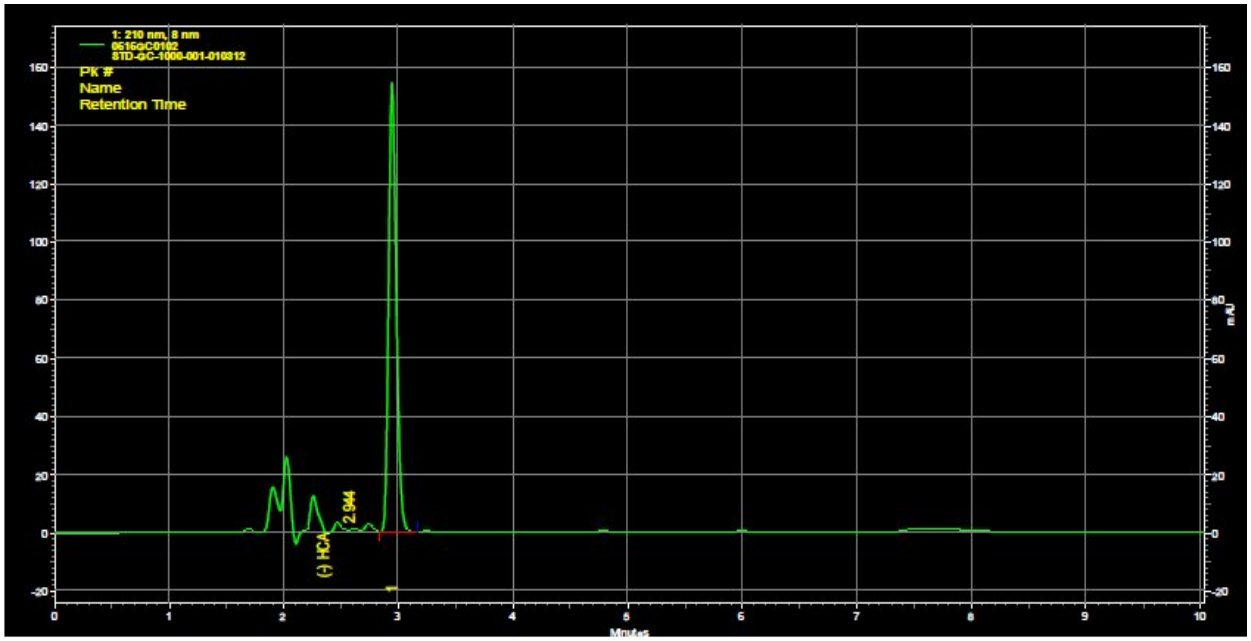


**Fig 1: Chromatograph of Garcinia cambogia standard**

| Peak | Name | Retention time | Height | Area | Area percent |
|---|---|---|---|---|---|
| 1 | HCA | 2.944min | 154128 | 711309 | 100 |

**Table 9: HPLC data of Garcinia cambogia standard**

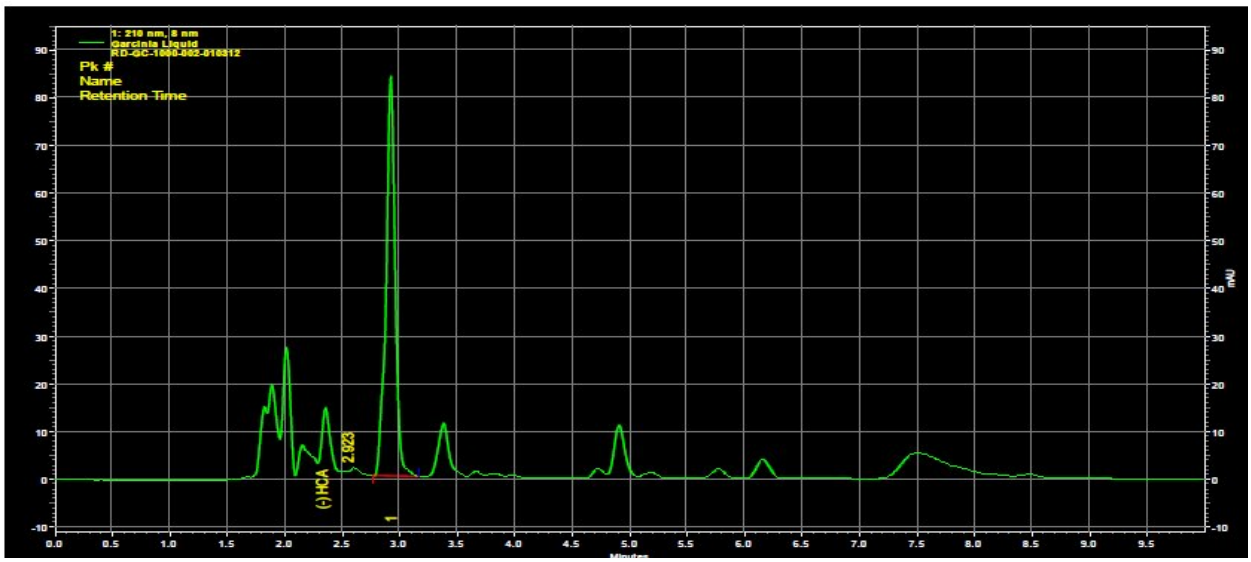


**Fig 2: Chromatograph of Garcinia cambogia formulation**

| Peak | Name | Retention time | Height | Area | Area percent |
|---|---|---|---|---|---|
| 1 | HCA | 2.923min | 83841 | 462941 | 100 |

**Table 10: HPLC data of Garcinia cambogia formulation**



eISSN: 2249 1023

**B. After stability study**

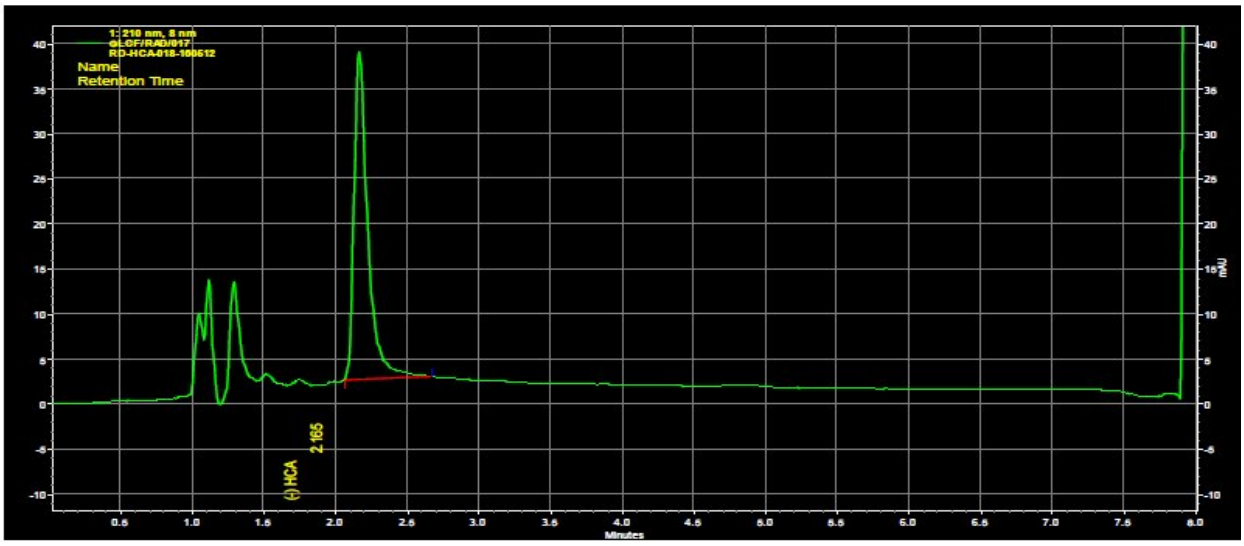


**Fig 3: Chromatograph of Garcinia cambogia formulation (stability study)**

| Peak | Name | Retention time | Height | Area | Area percent |
|---|---|---|---|---|---|
| 1 | HCA | 2.165 min | 36271 | 243383 | 100 |

**Table 11: HPLC data of Garcinia cambogia formulation (stability study)**

**L- Carnitine: by HPLC**

**A. Before stability study**

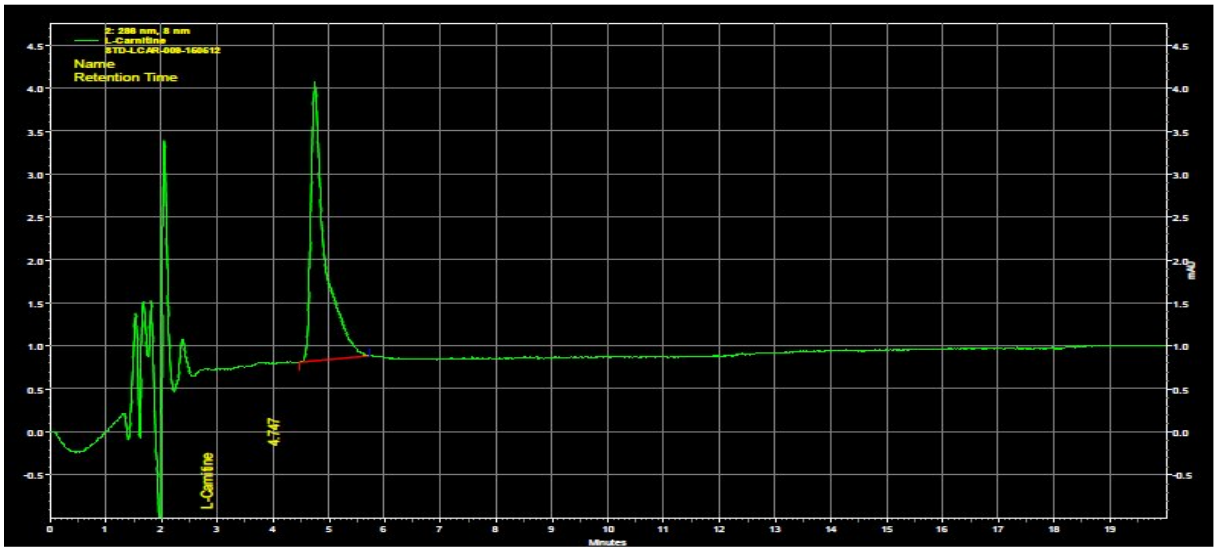


**Fig 4: Chromatograph of L-Carnitine standard**

| Peak | Name | Retention time | Height | Area | Area Percent |
|---|---|---|---|---|---|
| 1 | L-Carnitine | 4.747 min | 3236 | 55544 | 100 |

**Table 12: HPLC data of L-Carnitine standard**



eISSN: 2249 1023

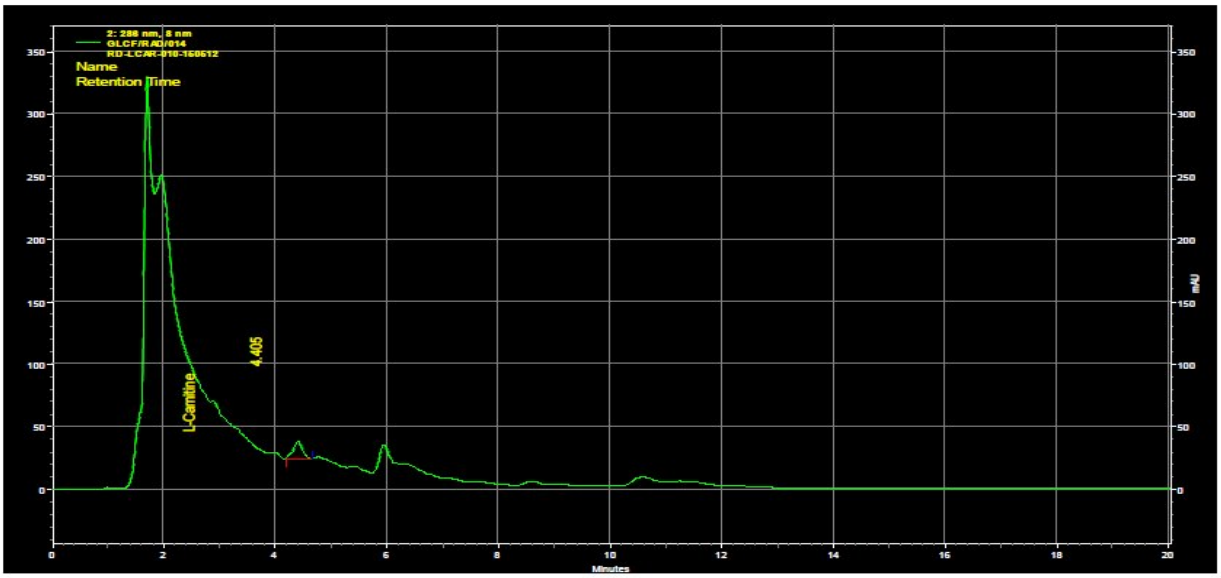


**Fig 5: Chromatograph of L-Carnitine formulation**

| Peak | Name | Retention time | Height | Area | Area Percent |
|---|---|---|---|---|---|
| 1 | L-Carnitine | 4.405 min | 13327 | 154124 | 100 |

**Table 13: HPLC data of L-Carnitine formulation**

**B. After stability study**

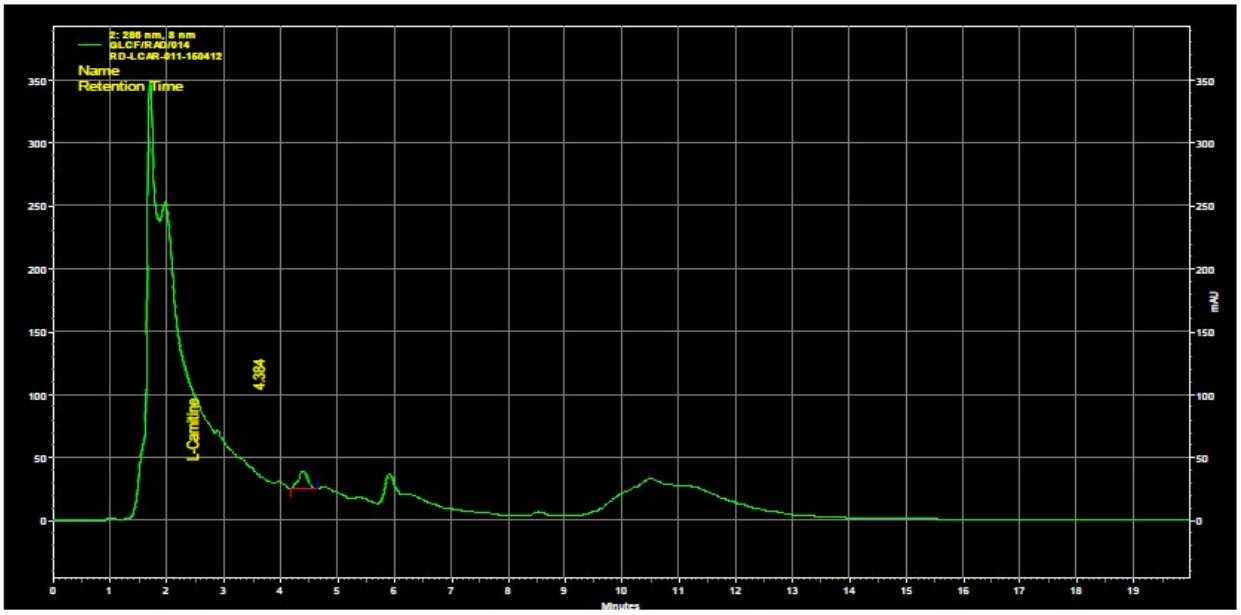


**Fig 6: Chromatograph of L-Carnitine formulation (stability study)**

| Peak | Name | Retention time | Height | Area | Area Percent |
|---|---|---|---|---|---|
| 1 | L-Carnitine | 4.405 min | 14153 | 160347 | 100 |

**Table 14: HPLC data of L-Carnitine formulation (stability study)**



eISSN: 2249 1023

**Chromium Picolinate: by AAS**

| Sr No | Conc. in µg/ml | Absorption at λmax: 357.9nm |
|---|---|---|
| 1 | 1 | 0.028 |
| 2 | 2 | 0.059 |
| 3 | 3 | 0.086 |
| 4 | 4 | 0.119 |

**Table 15: Calibration curve of Potassium dichromate**

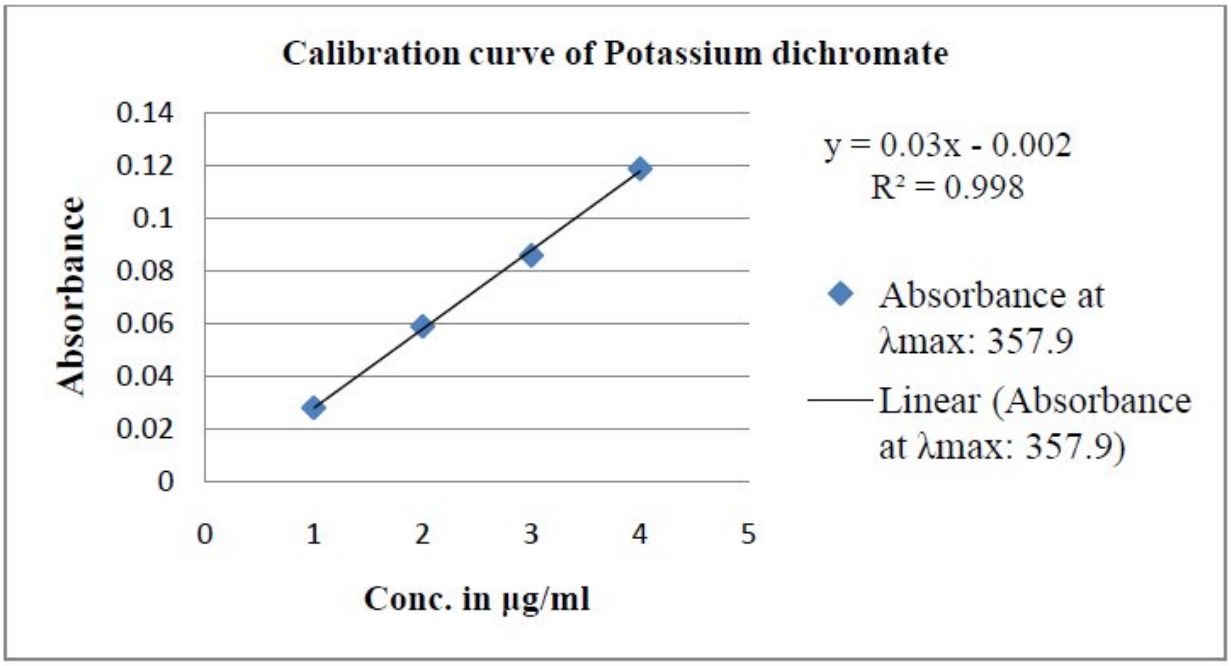


**Fig 7: Calibration curve of Potassium Dichromate**

**A. Before stability study:**

Absorbance of sample: 0.072

**Calculation:**

From the calibration curve:

**y = 0.03x - 0.002**

Where y= absorbance= 0.072

x= conc. in µg/ml =2.466 µg/ml

Calculated the quantity in mg of $C_{18}H_{12}N_3O_6Cr$ in the portion of chromium picolinate taken by the formula:

**(418.31/51.996) x (10 C)**

$C_{18}H_{12}N_3O_6Cr$ in the portion of chromium picolinate = 1.984 mg of $C_{18}H_{12}N_3O_6Cr$

2 mg of $C_{18}H_{12}N_3O_6Cr$ = 100% of $C_{18}H_{12}N_3O_6Cr$

1.984 mg of $C_{18}H_{12}N_3O_6Cr$ = 99.2 % of $C_{18}H_{12}N_3O_6Cr$

**B. After stability study:**

Absorbance of sample: 0.071

Calculation: From the calibration curve:

**y = 0.03x - 0.002**

Where y= absorbance= 0.072

x= conc. in µg/ml = 2.433 µg/ml

Calculated the quantity in mg of $C_{18}H_{12}N_3O_6Cr$ in the portion of chromium picolinate taken by the formula:

**(418.31/51.996) x (10 C)**

$C_{18}H_{12}N_3O_6Cr$ in the portion of chromium picolinate = 1.957 mg of $C_{18}H_{12}N_3O_6Cr$

2 mg of $C_{18}H_{12}N_3O_6Cr$ = 100% of $C_{18}H_{12}N_3O_6Cr$

1.984 mg of $C_{18}H_{12}N_3O_6Cr$ = 97.8 % of $C_{18}H_{12}N_3O_6Cr$



eISSN: 2249 1023

| Ingredients | Before stability | After stability |
|---|---|---|
| Garcinia Cambogia | 108.23%HCA | 108.16%HCA |
| L-Carnitine | 106.18% | 105.58% |
| Chromium picolinate | 99.2% | 97.8% |

**Table 16: Results of assay of APIs**

Under the solvent system 0.2 % phosphoric acid and Acetonitrile, the retention time of HCA standard was found to be 2.944 min and peak area 711309. The peak area for sample was found to be 462941 before stability study and 243383 after stability study for HCA in Garcinia cambogia extract. The purity of HCA standard is 62% in Garcinia cambogia. From the calculations 108.23% HCA before stability and 108.16 %HCA after stability study were found. So the Garcinia cambogia extract is stable in the formulation.

For L-Carnitine 0.1 M ammonium acetate and Acetonitrile (90:10) was used as mobile phase system. The retention time for standard L-Carnitine was found to be 4.747 min and peak area was 55544 for standard. The peak area for sample before stability and after stability was found to be 154124 and 160347 respectably. The purity of the L-Carnitine is 99% in standard. From the calculations 106.18% L-Carnitine before stability and 105.58 % L-Carnitine after stability study were found. So L-Carnitine is stable in the formulation.

Chromium picolinate was assayed by AAS method as per USP. Potassium dichromate was used as standard. From the standard calibration curve good linearity was obtained with $R^2$ value as 0.998. The absorbance of chromium in sample at 357.9 nm before and after stability was found to be 0.71 and 0.72 respectively.

From the calculations, the concentration of chromium picolinate in the sample before and after stability was found to be 99.2% and 97.8% respectively. Hence chromium picolinate is stable in the formulation.

The purpose of the present study was to develop and characterize a formulation of Garcinia cambogia, L-Carnitine and Chromium picolinate. Various formulation trials were taken. In these trials excipients ratio was varied and the effect of diluents base on the physical and microbial stability of formulation were studied. The prepared formulation was then packed in a suitable packing system. The pack integrity and efficiency were considered and successfully tested. The packed batches were then studied for accelerated stability conditions. Based on the data evaluated from the accelerated stability studies, long term stability of the developed product was evaluated and it was concluded that the formulated product would maintain its integrity and performance over the shelf life. In order to study the performance of the prepared product; a suitable analytical method was developed. For herbal formulation, microbiological tests are important for their stability and shelf life of the formulation. Assay was done for the active ingredient in the formulation.

## CONCLUSION

In the present study, three APIs were chosen Garcinia cambogia extract, L-Carnitine and Chromium picolinate, for the development of formulation and all have anti-obesity effects.It is generally recognized that monitoring the quality of any herbs and its formulations, chromatographic methods such as HPLC and HPTLC are ideal, which involves comparison between standard and sample.

From the results, it is concluded that prepared formulation is stable microbiologically by the use of suitable antimicrobial preservatives, methyl and propyl paraben, in formulations.

In future, this current research might give a path to develop and standardize antiobesity herbal formulation with maximum weight loss without any side effects and improved patient compliance.

## REFERENCES

1. Tripathi KD. 2003. Essentials of Medical Pharmacology. 5th edition. New Delhi: Jaypee brothers medical publishers 130-131.
2. Obesity information, Medical News, November2011 http://www.medicalnewstoday.com/info/obesity/



eISSN: 2249 1023

3. Lachman L, Lieberman HA, Kanic JL. 1987. The theory and Practice of Industrial Pharmacy; 3rd edition, Bombay, Vargeshe Publishing House, 458,488-493.
4. Martin A. 1993. Physical Pharmacy, 4th edition, Noida, B.I. publications, 366,461-462.
5. United States Pharmacopoeia and National Formulary, (24th) Asian Edition, The United States Pharmacopoeia Convention Inc., U.S.A., 909-910, 2462.
6. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy, 23rd edition, Pune, Nirali Prakashan, 111-112.
7. Quality Control Methods for Medicinal Plant Materials, 2004, WHO Geneva: Indian edition, 28-37.
8. Monika Mo, Andreas Kie, and Heinz Lo. Current Methods for Determination of L-Carnitine and Acylcarnitines, Monatshefte fur Chemie. 2005.136, 1279–1291.
9. Qing-Ri Cao, Shan Ren, Mi-Jin Park *et al.* Determination of Highly Soluble L-Carnitine in Biological Samples by Reverse Phase High Performance Liquid Chromatography with Fluorescent Derivatization, Archives of Pharmacal Research, 2007, 30, 1041-1046.

*Indo Global Journal of Pharmaceutical Sciences( ISSN 2249 1023 ; CODEN- IGJPAI) indexed and abstracted in EMBASE(Elsevier), SCIRUS(Elsevier),CABI, CAB Abstracts, Chemical Abstract Services(CAS), American Chemical Society(ACS), Index Copernicus, EBSCO, DOAJ, Google Scholar and many more. For further details, visit http://iglobaljournal.com*



**eISSN: 2249 1023**