Method Validation for Hydroxycitric Acid Lactone from Garcinia Indica by Hplc Methods

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Abstract: A Simple Hplc (High Performance Liquid Chromatography) Method For The Validation Of Hca In Garcinia Indica Has Been Developed. Hca Is Major Acid Component Was Shown To Be Potent Inhibitor Of Alpha Amylase And Alpha Glucosidase Leading To Reduction Of Carbohydrate Metabolism, Results In Weight Loss.Hca Inhibits Atp–Citrate Lyase, A Key Enzyme Responsible For Promoting Fat Synthesis. As A Result Of This Inhibition, Energy Is Di-Verted For The Production Of Glycogen In Liver And Muscles, Instead Of Fat Synthesis. Since Higher Glycogen Levels Signal Satiety To The Brain, Hca Suppresses Appetite. As A Direct Result Of Atp–Citrate Lyase Inhibition, Malonyl-Coa Is Not Produced, Which Indirectly Helps In Burning The Fat. The Study Of The Mechanism Of Action Of Hcal Would Be Difficult For The Reason That It Exists In Equilibrium With Hca Under Aqueous/Acidic Conditions. Hcal Shows Better Activity In Terms Of Appetite Suppression And Reduction In Weight Gain And This May Be Due To Continuous Conversion Of Hcal Into Hca As It Gets Utilized By The Body. Hcal May Thus Act As A Pro- Drug. Once Hcal Gets Converted Into Hca It Follows The Same Mechanism Of Action As Hca In Reducing Body Weight Gain And Appetite Suppression.

Key Words: ***Garcinia Indica; (−)-Hydroxycitric Acid; Antiobesity***

Introduction

Garcinia Lndica (Hindi – Kokam) A Slender Evergreen Tree With Drooping Branches, Leaves Ovate Or Oblong Lanceolate, 2.5 - 3.5 Inch Long And 1 - 1.5 Inch Broad, Dark Green Above And Pale Beneath, Fruits Globose Or Spherical 1 - 1.5 Inch Diameter, Dark Purple When Ripe Enclosing 5 - 8 Large Seeds. The Tree Is Found In Tropical Rain Forests Of Western Ghats, From Konkan Southwards In Mysore, Coorg And Wynaad. The Fruit Has An Agreeable Flavour And A Sweetish Acid Taste. It Is Used In Konkan Chiefly In The Form Of Kokam Prepared By Drying The Outer Rind, Soaking It Repeatedly In The Juice Of The Pulp And Sun-Drying. Kokam Contains Approximately 10% Malic Acid And A Little Tartaric Or Citric Acid. The Fruit Of The Garcinia Lndica Is Anthelmintic And Cardiotonic And Useful In Piles, Dysentery, Tumours, Pains And Heart Complaints. The Fruit Rind Of Garcinia Mica Also Contains Hydroxy Citric Acid [Krishnamurthy Et A/. 19821. The Seeds Of The Fruit Yield (23 - 26% On The Weight Of Seed, And 44% On The Weight Of Kernels) A Valuable Edible Fat Known In Commerce As Kokam Butter. Kokam Butter, Like Other Garcinia Fats, Is Rich In Combined Stearic And Olelc Aclds. It Contains About 75% Of Mono-Oleodisaturated Glycerides And Possesses A Fairly Low Melting Point. Kokam Butter Is Considered To Be Nutritive, Demulcent, Astringent And Emollient. It Is Suitable For Ointments, Suppositories And Other Pharmaceutical Purposes. It Is Used As A Local Application For Ulcerations And Fissures Of Lips, Hands Etc.

Hydroxycitric Acid (Hca) Is A Constituent Of Garcinia Cambogia, Garcinia Indica And Garcinia Atroviridis, Which Is Widely Used In Food Preparation As A Soaring Agent And Is Known To Cause Appetite Suppression And Showed That Hca Was A Potent Competitive Inhibitor Of The Extra-Mitochondrial Enzyme Adenosine Triphosphate Citrate (Pro-3s)-Lyase. Subsequently In Vitro And In-Vivo Studies Revealed That Hca Not Only Inhibited The Action Of Citrate Lyase And Suppressed De Novo Fatty Acid Synthesis (Lowenstein, 1971) But Also Increased Rates Of Hepatic Glycogen Synthesis (Sullivan, Triscari, & Neal Miller, 1974), Suppressed Food Intake (Sullivan, Triscari, Hamilton, & Neal Miller, 1973) And Decreased Body Weight Gain (Nageswara Rao & Sakeriak, 1988). Recently It Has Been Established That A High Dose Of Hca Was Effective In Suppressing Fat Accumulation In Developing Male Zucker Obese Rats But Was Found To Be Highly Toxic To The Testis (Hayamizu Et Al., 2003; Saito Et Al., 2005). Various Salts Of This Naturally Occurring Hca Are Now Being Used In Nutraceuticals And Incorporated Into Many Preparations In Weight Management And Are Sold Over-The-Counter To Consumers All Over The World.

Its Preparation Involves Water Extraction Of Garcinia Rind, Followed By Purification Using Ion-Exchange Resins And Converting It Into Different Salts. Many Of The Hca Salts Currently Available Are Crude Preparations With No Definite Metal Content. In The Context Of Stringent Regulations Being Enforced On The Nutraceuticals With Respect To Quality And Reproducibility, It Is Relevant To Investigate New Methodologies In Order To Make Pure Products With Definite Composition. Efforts To Make These Salts With Defined Purity/Composition Resulted In The Isolation Of Pure Crystalline Hcal.

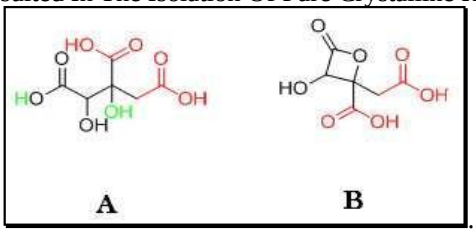

Figure-1 Structural Representation Of (A)Hydroxycitric Acid And (B) Hydroxycitric Acid Lactone**.**

Materials And Methods-

***Plant Material*-**Fruits And Fruit Pericarps Of *G. Indica* Were Collected From Bangalore And Leaves, Stem Barks,Seeds Were Collected From Dapoli, Maharashtra,India. (S.K.Et. Al. 2013)

***Chemicals And Standards*-**Hcal Was Procured From Life Technologies (India) Pvt. Ltd. And Their Purity Was Above 99.5% By Hplc. (S.K.Et. Al. 2013)[4]

Sample Preparation-

***Leaves*-** About 20 G Of Fresh Leaves Of G. Indica Were Extracted With 250 Ml Of Water At 15 Lbs/In.2 Pressure For 20 Min And Filtered. The Extraction And Filtration Was Repeated Twice For Complete Extraction Of The Organic Acids. The Extract Was Concentrated To 50 Ml Under Vacuum And Was Treated With 200 Ml Of Ethanol To Remove Pectinaceous Material And Centrifuged. The Supernatant Was Concentrated Under Reduced Pressure To 25 Ml And Stored At 4 °C Until Further Use. The Acid Content Was Found To Be 5.88–6.29% (W/W) As Determined By Acid–Base Titration Using Phenolphthalein Indicator.

***Rinds*-**About 10 G Of G. Indica Rinds Were Extracted With 50 Ml Of Water At 15 Lbs/In.2 Pressure For 20 Min And Filtered. The Extraction And Filtration Was Repeated Twice For Complete Extraction Of The Organic Acids. The Extract Was Decolorized Using Activated Charcoal And Filtered. The Decolorized Extract Was Concentrated To 25 Ml Under Vacuum And Was Treated With 100 Ml Of Ethanol To Remove Pectinaceous Material And Centrifuged. The Supernatant Was Concentrated Under Reduced Pressure To 25 Ml And Stored At 4 °C Until Further Use. The Acid Content Was Found To Be 12.5–15.1% (W/W) With Respect To Weight Of Rinds As Determined By Acid–Base Titration.

Validation Of Hplc Method-

***Calibration And Linearity*-**

The Linearity Of The Method Was Evaluated By Analyzing A Series Of Hca Standards. About 10 µl Of Each Of The Five Working Standard Solutions Containing 2–10 µg Of Free Hca Was Injected OnTo The Hplc And Elution Was Carried Out As Discussed Above And Peak Area Responses Were Obtained. The Calibration Curve For Hca Was Prepared By Plotting Concentration Of Hca VerSus Peak Area (Average Of Three Runs).

***Range*-**

The Calibration Range Was Established Through Consideration Of The Practical Range Necessary According To The Use Of The Hca Concentration Present In The Samples. This Range Includes Concentrations From Lower Limit Of Concentration (Lloq) To The Upper Limit Of Quantification(Uloq).

***Determination Of The Limit Of Quantification*-**

The Limit Of Quantification (Loq) Was DefinedAs The Lowest Hca Concentration, Which Can Be Determined With An Accuracy And Precision
<20%.

***Quantification Of Organic Acids In Samples*-**

A Known Volume Of (20 µl) Of The Sample Prepared Above Was Injected Into The Hplc And Concentration Of Organic Acids Was Obtained Di-Rectly From The Peak Area And By Application OfThe Dilution Factor. The Organic Acids In The Sam- Ples Were Expressed As G/100 G Of Sample.

***Recovery Of Hca*-**

The Organic Acids Of Selected Samples Of *G. Indica* Leaves And Rinds Were Approximately Doubled By Spiking With Known Amounts Of Hca. The Spiked Samples Were Prepared For Acid Determination As Described In The Sample Preparation Section And Were Analyzed.

Results And Discussion-

Hplc Method Was Carried Out Using Different Mobile Phases I.E. 6, 8, 10, 12 Mm H_2so_4. It Was Found That Is The Best Solvent For The Separation Of All Peaks From *G. Indica* Samples. Before Actual Extraction Solvent Was Chosen, Preliminary Studies Were Performed With Different Solvents Like Ace- Tone And Methanol [6]. It Was Concluded That Aqueous Extraction Yielded Maximum Yield Of Or- Ganic Acids.

Hca Was Resolved As Single Peak In All Samples Analyzed With No Interference From Other Com- Pounds. The Identity Of The Hca Peak Was Confirmed By Determination Of Retention Time And By Spiking With Standard Hca. The Retention TimesOf The Hca Lactone, Hca And Citric Acid In All Samples Were Found To Be 4.69±0.28, 5.02±0.19 And 5.75±0.03 Min, Respectively.

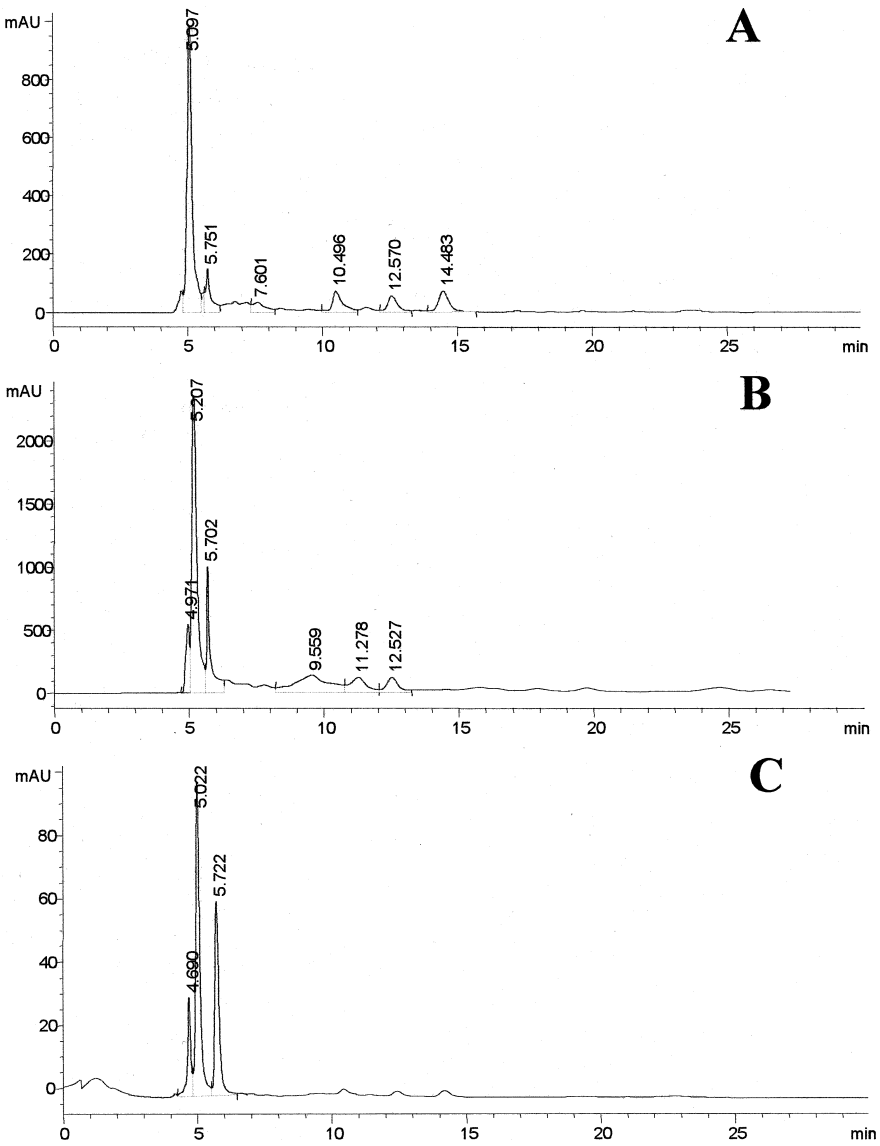

Fig. 2. Hplc Chromatograms Of (A) Rinds; (B) Leaves; And (C) Standard Hca Lactone, Hca And Citric Acid.

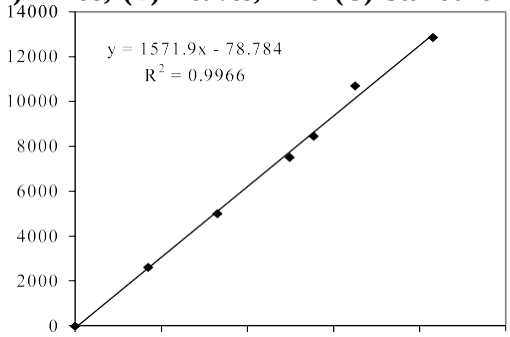

Fig. 3. The Linear Relationship Between The Area And Concentration Of Hca.

A Calibration Curve Was Derived From Three Injections Of Six Concentrations Of Hca. Linearity Was Found In The 2 – 9 µg Concentration Range And It Has A Good Reproducibility And Accuracy (Fig.3). The Following Regression Equation Was Obtained Y =1571.9x−78.784, Where Y Is The Peak Area And X Is The Concentration Of Hca. The Correlation Coefficient Of The Calibration Graph Was ≥0.9966. The Estimated Loq In This Study Was Found To Be 1.4 µg.

To Determine The Recovery And To Ensure TheValidity And Reproducibility Of The Proposed Method, Repeated Injections Of The Same Samples Of Each Of The Studied *G. Indica* Rinds And Leaves Were Used. These Samples Were Prepared By Addi- Tion Of Known Amount Of Standard Hca To Exact Weights Of Previously Assayed Rinds And Leaves. The Obtained Results Indicated The Hca Was Al- Most Quantitatively Recovered From The Three Stud- Ied Samples. The Recoveries Of Hca From Different Samples Are Presented In Table 2. They Ranged From 93.5 ±3.9 To 96.9 ±2.7% Emphasiz- Ing The Accuracy Of The Method. The CoefficientsOf Variation Resulting From Three DeterminationsWere 2.8−4.2% Indicating The Precision Of The Method.

Recovery Of Hca From Kokam Rinds And Leaves*

Samples	% Recovery	% Cv
Rinds-I	95.0 ±3.4	3.6
Rinds-Ii	93.9 ±3.2	3.4
Rinds-Iii	93.5 ±3.9	4.2
Leaves-I	95.9 ±2.7	3.0
Leaves-Ii	96.9 ±2.7	2.8
Leaves-Iii	93.6 ±3.4	3.6

Cv, Coefficient Of Variation; *N=3.

Conclusion-The Present Method Is Simple And Accurate For The Determination Of Organic Acids In Leaves And Rinds Of *G. Indica* By Hplc. Values Found By The Titration And Hplc Methods Were Comparable But Were 0.1 – 1.3% Higher Than The Total Acid By Hplc. The Coefficients Of Variation Were 2.8 – 4.2% Indicating The Precision Of The Method. Fi-Nally This Method Can Be Used An ExcellentAlternate To Glc And Titration Method For The Estimation Of Hca In Dilute Extracts. Reproduci- Bility, Accuracy And Sensitivity Of The Method Are Satisfactory. This Method May Be Considered For Routine Analysis Of Large Number Of Samples Of *G. Indica*.

Reference-

1. Vipul Upadhyay , Amit Tiwari, Neeru Sharma, H.M.Joshi ,Brijpal Singh, "Rapid Determination & Standardization Of Garcinia Fruit Extract Of Hydroxycitric Acid (Hca) In Garcinia Cambogia By Hplc", Pharmbit, Jan – Jun 2013; Xxvii (1).
2. G. Venkateswara Rao, A.C. Karunakara, "Hydroxycitric Acid Lactone And Its Salts: Preparation And AppetiteSuppression Studies", Food Chemistry 120 (2010) 235-239.
3. Krishnamurthy N, Lewis Y. S And Ravindranath 8.(1982). Chemical Constituents Of Kokkum Fruit Rind. Journal Of Food Sciences And Technology, 19,97-100.
4. S.Kumar, S. Sharma And S.K.Chattopadhyay, Rapid And Sensitive Hplc-Pda Method For Simultaneous Identification And Quantification Of Dietary Weight Reducing Compound Hydroxy Citric Acid Lactone And Chemo Preventive Compounds Isoxanthochymol And Xanthochymol In *Garcinia Indica*, International Food Research Journal 20(1): 397-402 (2013).
5. G.K. Jayaprakasha , K.K. Sakariah, Determination Of Organic Acids In Leaves And Rinds Of *Garcinia Indica* (Desr.) By Lc, Journal Of Pharmaceutical And Biomedical Analysis 28 (2002) 379 – 384.
6. Gogoi Et Al.,Estimation Of (-)-Hydroxycitric Acid (Hca) In Garcinia Lanceaefolia Roxb. Using Novel Hplc Methodology, Ijpsr, 2014; Vol. 5(11): 4995-4999.
7. Antony B., Varghese, W. And Merina, E. 2004. PreparationAnd Evaluation Of Hydroxyl Citric Acid From *Garcinia Cambogia* Extract Using Rp Amide Hplc. Indian Journal Of Pharmaceutical Sciences 66: 208-211.
8. E.Thom (1996). (-) Hydroxycitrate In The Treatment Of Obesity. *International Journal Of Obesity, 20*, 48.