December 2008 Volume 7 Issue 12

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 7(12) 2008 [854-856]

Estimation of hydroxycitric acid (-)-hca in garcinia indica choisy by HPLC method

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Received: 8th November, 2008 ; Accepted: 13th November, 2008

ABSTRACT

A simple, fast and precise reverse phase High performance liquid chromatographic isocratic method has been developed. The aim of the study was to develop a High performance liquid chromatography (HPLC) assay method for accurate determination of (-)-HCA in *Garcinia Indica choisy*. (-)-Hydroxycitric acid is the principal acid of fruit rinds of *Garcinia* cambogia and *Garcinia* Indica. The experimental procedure involved Diode Array Detector (at 210nm), Mobile phase (0.01M Potassium dihydrogen orthophosphate in purified water and adjust the pH to 2.80 with orthophosphoric acid, with the flow rate 1.0ml/min) and Inertsil ODS 3V (250× 4.6mm, 5µ size) column. A calibration curve of mean peak area vs. concentration showed good linearity. The linearity range selected for the experiment is from 50% to 150% of the actual concentration level i.e. 50ppm to 150ppm.The active ingredient , Hydroxy citric acid(-)-), is weight loss promoter extracted from the rind of the fruit. The fruit rinds of *Garcinia Indica* contain 8% (-)-HCA.

KEYWORDS

Hydroxy citric acid;
HPLC;
Reverse phase;
Garcinia Indica choisy.

INTRODUCTION

Garcinia (family: Guttiferae) is a large genus of polygamous trees distributed in tropical Asia and Africa. It consists of 180 species, of which 30 species are found in India. *Garcinia Indica* is a slender evergreen tree with drooping branches; its leaves are oval or oblong 2.5- 3.5 inches long and 1-1.5 inches broad, dark green above and pale green beneath; its fruits are globose or spherical, 1-1.5inches Diameter, dark purple when it rips and enclosing five to eight large seeds. The tree is found in the tropical rain forests of Western Ghats, from southward Konkan to Mysore. It flowers in November-February and fruits ripes in April- May.

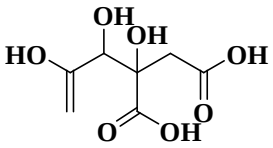
However, the isolation of (-)-Hydroxycitric acid [(-)-HCA] from a few species of *Garcinia* and its biological properties have attracted the attention of biochemists. Citric acid is a substance involved in the metabolism of carbohydrates. (-)-HCA (a modified form of citric acid) is believed by some to inhibit the enzyme that allows carbohydrates to be stored as fat. In the presence (-)-HCA, excess carbohydrates would be expended instead of being stored as fat. A decrease in appetite is purported to be a side effect of this process, which further promotes weight loss.

The proposed method is cost effective as the buffer used is of the low cost and easily available.

Chemical Name

1,2-Dihydroxy-1,2,3-propanetricarboxylic acid

Structure



Chemical and reagents

Potassium dihydrogen orthophosphate AR grade, ortho phosphoric acid AR grade (88%), Milli-Q water were used. Nylon filter of pore size 0.45m was obtained from Sartorius. Standard (-)- HCA (VMSRF).

Garcinia Indica choisy-was collected in May, from Ratnagiri district and stored at refrigerator. The dried sample was chopped into small parts with a blender.

Instrumentation and chromatographic conditions

HPLC system of Waters Alliance make was used, which was equipped with separation module 2695, PDA detector 2996 and column compartment. Data acquisition and data analysis was done using Empower software. An Inertsil ODS 3V column (250mm × 4.6mm) having particle size 5µ was used as stationary phase. The detection was set at 210nm. The flow rate was 1.0ml/min.

Preparation of buffer

Mobile phase: (0.01M Potassium dihydrogen orthophosphate

In purified water and adjusted the pH to 2.80 with orthophosphoric acid

Diluent: Ethanol: Hydrochloric acid (99:1)

Standard solution

Hydroxy citric acid stock solution

Dissolved 25mg of (-)-Hydroxycitric acid working standard in 25ml volumetric flask with diluent. Dilute 5ml of above solution to 50ml with diluent.

Sample solution preparation

The fruit was well dried and then ground to powder. About 10 gm of the powder was extracted in 200ml of 95% Ethanol and extracted in a soxhlet extract. The materials were then filtered using Whatman No.1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in fridge prior to use.

Procedure

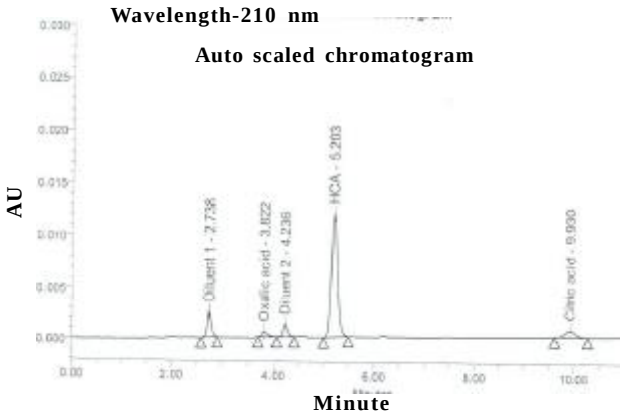


Figure 1

Inject 20µl each of diluent, six replicates injection of Standard solution and sample solution twice in the chromatograph.

The system suitability was confirmed by Relative standard deviation for peak area of (-) - Hydroxycitric acid of six standard injections NMT 2.0%.

RESULTS AND DISCUSSION

Method development

The method described herein was developed for the estimation of the of (-)- Hydroxycitric acid. Also references listed in this paper were taken as a base. Wavelength was selected by scanning standard over the range of wavelength 200nm to 400nm.Hydroxy citric acid show UV maxima at 210nm, also the good peak response was observed.

Method validation

System suitability

The system suitability was studied from the injection of the standard solution.

The Relative standard deviation for peak area of (-)-Hydroxycitric acid of six standard injections was 0.56%.

Recovery study

The experiment was studied at 80%, 100% and 120% level of the working concentration of Hydroxycitric acid. Recovery results

Precision

Injection repeatability

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Level	Wt. in mg	Amount added in ppm	Amount recoverd in ppm	% Recovery
80	16.23	81.15	82.78	102.00
100	20.32	101.6	102.42	100.80
120	24.19	120.95	103.42	102.04
		Mean recovery		101.60
		SD		0.70
		%RSD		0.69

Injection repeatability was assessed by performing replicate injections (n = 6) of standard preparation. Relative Standard Deviation of injection precision was 0.06%.

Sample repeatability

The sample precision was studied by analyzing the same sample for six times and calculating the % assay.

The % RSD for Hydroxycitric acid content was 1.7% which within limit Not more than 2%.

Standard and sample solution stability

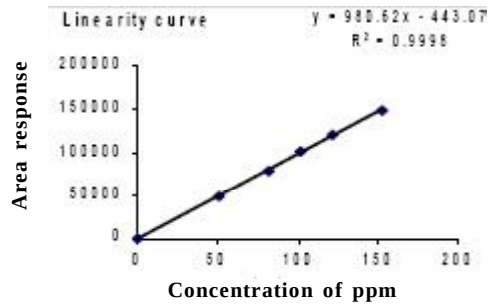
Solution stability of standard solution and sample solution of Hydroxycitric acid and *Garcinia Indica choisy* was studied by repetitive injection at regular time interval and was found to be stable for 48 hrs.

Linearity of the detector response

In this study the linearity range selected for the experiment is from 50% to 150% of the actual concentration level i.e. 50ppm to 150ppm. Five levels were prepared and each level was injected in duplicate into the chromatographic system. Mean peak area of each level was calculated. A graph of mean area vs. concentration was plotted and the best fit line was determined by linear regression. % intercept and Correlation coefficients ® was calculated of the same was calculated.

Linearity results

Concentration in ppm	Area response
50.80	48251
81.28	79036
101.60	100184
121.92	119504
152.40	148522
Correlation coefficient	0.9998
Slope	980.62
Intercept	-443.07



CONCLUSION

The method is very simple and rapid. High percentage of recovery indicates that the method is free from interference. Method was validated for its performance parameters such as Specificity, Linearity and range, Recovery, solution stability, Precision and Ruggedness was found to be specific and reliable for its intended use.

ACKNOWLEDGMENTS

The author thanks Ivax India Pvt Ltd., for providing the laboratory facility.

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