RESEARCH PAPER

Accepted 17 February 2004
Revised 24 November 2003
Received 11 April 2003
Indian J. Pharm. Sci., 2004, 66(2): 208-211

Preparation and Evaluation of Hydroxy Citric Acid from *Garcinia cambogia* Extract using RP-amide HPLC

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***Garcinia cambogia* is an exotic fruit grown in the southern parts and Western ghats of India. Hydroxy citric acid is the active component present in this fruit which imparts the characteristic sour taste. Hydroxy citric acid is found to be physiologically active. It is a significant factor in reducing obesity. Hydroxy citric acid is an alpha, beta dihydroxy tricarboxylic acid, which is less stable and easily converted into its lactone. Both hydroxy citric acid and its lactone are estimated using RP amide C_{16} HPLC column and is described in this paper. Ethylene diamine salt of hydroxy citric acid is used as the reference material. The percentage of hydroxy citric acid varies from 45-65% in different salts of hydroxy citric acid.**

Garcinia cambogia is a tropical plant found in the Western Ghats of India and in many parts of Sri Lanka[1]. The fruit of this plant commonly known as 'Malabar tamarind' is used traditionally for cooking especially fish[2]. *Garcinia cambogia* is grown mainly as wild and some hybrid varieties are cultivated mainly for its market potential.

Scientific data has shown that hydroxy citric acid (HCA) is the active ingredient of *Garcinia cambogia* fruits, which also imparts its sour taste[1]. Hydroxy citric acid was wrongly identified as citric acid and tartaric acid[3]. HCA is found to inhibit fatty acid synthesis by rat liver *in vivo* and by perfused liver[4-5]. It also inhibits β-hydroxysterol synthesis and also fatty acid synthesis in rat brain[6-7]. Hydroxy citrate inactivates citrate lyase, citrate synthase and ATP citrate lyase[8].

HCA contains an additional -OH group compared to citric acid. As the carbon atom at position 2 is chiral, different isomers are possible for hydroxy citric acid[9]. Presence of these two -OH groups and three carboxylic groups make the molecule unstable. It is easily liable to form lactones[10].

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Hydroxy citric acid can be estimated by a spectrophotometric method[11] and also by a HPLC method[12,13]. Even though different types of products containing *Garcinia cambogia* extract are available in the market (tablets, capsules, chewing gums, soft drinks, biscuits and mouth sprays) an officially accepted assay method is not available. Reference standards from established chemical houses are also not available. These limiting factors make the estimation of hydroxy citric acid difficult. This paper describes the estimation of HCA using RP amide C_{16} column in HPLC system. Being an alpha, beta dihydroxy acid, HCA is easily converted into its corresponding lactone. Lactones give separate peak on HPLC. However, lactones give no colour reaction with meta vanadate[10]. This differentiates HCA and its lactone. In this case by using the RP amide column, the lactones can also be estimated. Presence of other alpha hydroxy acids such as tartaric acid and citric acid present if any can also be determined by this method.

MATERIALS AND METHODS

Dried fruits of *Garcinia cambogia* were used for the analysis which were collected from Konkan area. These raw materials were labeled as GCRM-1, GCRM-2, GCRM-3, GCRM-4 and GCRM-5. Ethylenediamine salt of HCA was

purchased from Fluka Chemie GmbH (Buchs, Switzerland). Secondary standard of HCA was collected from Transglobal Resource Inc., Seattle, USA. All solvents used for the preparation and analysis were of AR grade. For HPLC, the solvent used was HPLC grade water. The chemicals used like L-aspartic acid, sodium hydroxide, calcium hydroxide, sulphuric acid, potassium hydroxide, sodium sulphate, acetic acid, citric acid, tartaric acid, lactic acid were purchased from Merck, Mumbai.

Preparation of *Garcinia cambogia* extract, hydroxy citrates and lactone:

Approximately 50 g of dried rinds of *Garcinia cambogia* were weighed into a 500 ml round bottom flask. Three hundred milliliters of distilled water was added and refluxed for 2 h in a boiling water bath. The extract was taken in a beaker, cooled, filtered and the filtrate collected. The residue was again refluxed with water. The process was repeated thrice. All the extracts were combined and concentrated upto 50% of the total dissolved solids level. This was the crude extract and the yield obtained was 23.5 g. To the crude extract in a 1000 ml beaker, a clear solution of calcium hydroxide was added drop by drop until the pH reached 8. Calcium hydroxy citrate was precipitated and collected by filtering under vacuum. This residue was washed with water to remove excess calcium hydroxide, if any. The washed material was dried in an air oven at 110° for about 4–6 h till the powder was free from moisture. The yield of calcium hydroxy citrate was about 10 g. Some modifications were made for the preparation of potassium hydroxy citrate hence this is a water soluble salt. Water extracts of *Garcinia cambogia* fruit rind (50 g) was prepared as described earlier. The combined extracts were concentrated upto 50% total dissolved solids level. This acidic extract was neutralised (pH–8) by adding 10% potassium hydroxide solution. Potassium hydroxy citrate was separated by adding alcohol and the yield obtained was 12.8 g. Sodium hydroxy citrate was prepared in a similar way used for the potassium hydroxy citrate. Here 10% solution of sodium hydroxide was used instead of potassium hydroxide. Using 50 g dried fruit rind, the product obtained was 11.5 g. HCA lactones were prepared by passing a known solution of potassium hydroxy citrate through a strong cation exchange column. The eluted solution was collected and concentrated. This contained about 20–25% lactones.

HPLC analysis:

Two hundred and fifty milligrams of the sample was accurately weighed out into a 100 ml volumetric flask. Then 5 ml water was added followed by 5 ml concentrated sulphuric acid. The material was dissolved and made upto the volume using HPLC grade water. The pH of the solution was measured using a pH meter.

Fifty milligrams of ethylenediamine salt of hydroxy citric acid (having 50% purity) was accurately weighed into a 50 ml volumetric flask. The material was dissolved in 5 ml 50% H_2SO_4 and made up to the volume by adding HPLC grade water. The resulting stock solution contained 500 µg/ml hydroxy citric acid. An internal standard solution was prepared by dissolving 50 mg of L-aspartic acid in 5 ml 50% H_2SO_4 and water in a 100 ml volumetric flask, to give 500 µg/ml concentration. Different concentrations of the standard solutions were prepared by taking different volumes of the stock solution. Three milliliters of internal standard solution was added and made upto 10 ml using HPLC grade water. From these 20 µl of the solution was injected into the HPLC column and regression graph was drawn. Similarly 20 µl of the sample solution was injected and the elution profile was noted. The elution profiles of aspartic acid, oxalic acid, tartaric acid and citric acid were also found out by preparing solutions of each acid in HPLC grade water. The solution was sonicated and filtered through 0.45 micron filter and 10–20 µl solution was injected into HPLC. The instrument used was HPLC class VP system supplied by Shimadzu and column used was RP amide C_{16} guard column having dimensions 2.5 cmx4.6 mmx0.5 µm. The mobile phase was 0.1 M sodium sulphate in HPLC water, pH adjusted to 2.1 with sulphuric acid, filtered, sonicated and maintained a flow rate of 0.5 ml/min. Ten to twenty microlitres of the sample solution was injected and detection was made at a wavelength of 203 nm. The concentration of HCA in the sample solution was calculated by checking the area of HCA peak of sample, standard and knowing the concentration of standard and sample. Concentration of HCA in the sample solution=Peak area of HCA sample x concentration of standard/Peak area of HCA standard x concentration of sample.

RESULTS AND DISCUSSION

HCA is the active component present in *Garcinia cambogia* extract. One among the limiting factors to use this product is the unavailability of a standard estimation procedure for HCA. Recently the spectrophotometric estimation procedure was published by Antony *et al.*[11]. HPLC method is also available.

Here the results obtained for the estimation of HCA in the *Garcinia cambogia* extract using C_{16} RP–amide HPLC

column are discussed. This column is very specific for alpha hydroxy acids. The elution profile of HCA present in calcium hydroxy citrate is shown in fig.1. The regression graph of ethylene diamine salt of HCA obtained was linear. The elution profile of other organic acids like aspartic acid, oxalic acid, tartaric acid and citric acid are shown in fig. 2. Fig. 3 shows the HPLC profile of HCA lactones. From these figures it was clear that HCA could be easily determined by HPLC using a C_{16} RP–amide column. It could also be identified from the lactones.

The separation was very clear for each acid. HCA content present in calcium hydroxy citrate was compared with spectrophotometric method and HPLC procedure using C_{16} RP–amide column. In the spectrophotometric method, calcium hydroxy citrate was converted into free acid by hydrolysis using dilute sulphuric acid which on colour reaction with sodium meta vanadate forms a colour complex having absorbance maxima at 467 nm. The comparative analysis report is shown in Table 1. From these results it was observed that the results were comparable and almost same.

The hydroxy citric acid present in *Garcinia* can be estimated by colorimetric method using UV spectrophotometer. This method is very simple and less time consuming[8]. The only draw back is its time dependence. The colour complex formed is unstable and changes its colour on keeping[9]. Even though the HPLC analysis is time consuming, it is more accurate and specific. We can draw the calibration graph automatically and find out the results. Determination of HCA by HPLC is also known[12]. According to one HPLC procedure, the results are obtained by a comparative method by calculating the total acids and by deducting the percentage of citric acid from the total acids. In the other method, the column used is C_{18} column. But in the procedure described here, the column used is C_{16} RP–amide HPLC column which is ideal for alpha hydroxy acids.

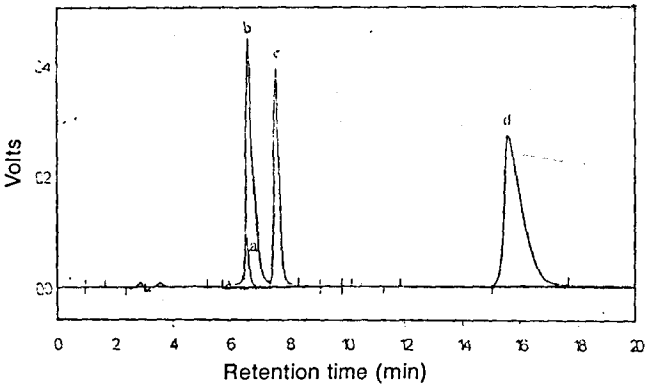

Fig. 2: Elution profile of aspartic acid, oxalic acid, tartaric acid, and citric acid using C_{16} RP–amide column.

The acid solutions prepared and injected into the C_{16} RP–amide column individually. The retention time noted. Then the solutions mixed and 20 µl was injected. The peaks obtained were, a – aspartic acid, b - oxalic acid, c - tartaric acid and d - citric acid.

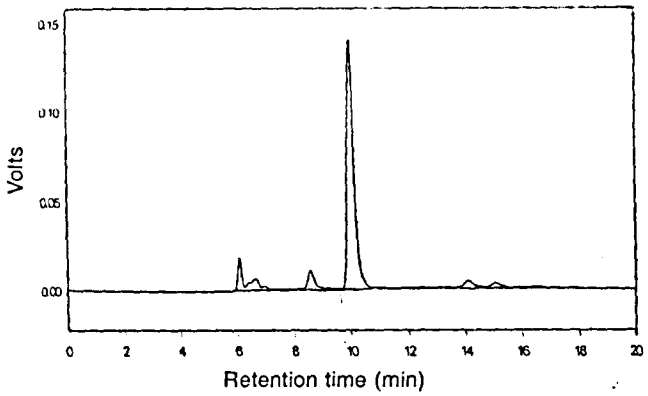

Fig. 1: Elution profile of calcium hydroxy citrate using C_{16} RP–amide HPLC column.

Calcium hydroxy citrate solution prepared as described in materials and methods. 30–40 µg material injected into C_{16} RP–amide column. Retention time and area percentage were noted.

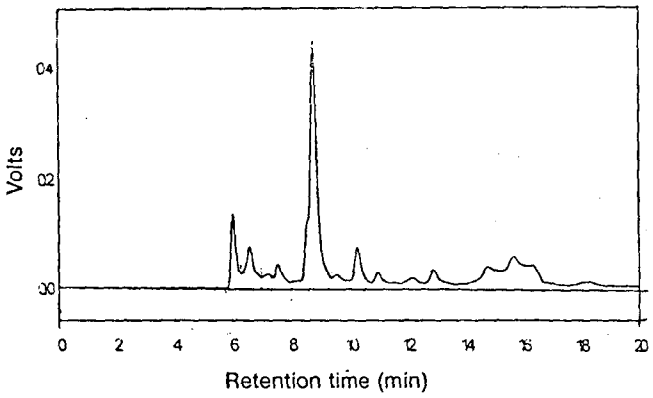

Fig. 3: Elution profile of lactone using C_{16} RP–amide column.

Hydroxy citric acid lactone prepared as described in materials and method. 160-170 µg sample injected into C_{16} RP–amide column. Retention time and area percentage noted.

TABLE 1: COMPARISON OF HCA BY SPECTROPHOTOMETRIC METHOD AND HPLC METHODS

Sample (Calcium hydroxy citrate)	UV method* (% of HCA)	HPLC method* (% of HCA)
GCRM – 1	50.8	50.9
GCRM – 2	51.0	51.2
GCRM – 3	50.3	50.6
GCRM – 4	52.0	52.3
GCRM – 5	52.5	52.9

*The results obtained are the average of three experiments. Calcium hydroxy citrate prepared from different raw materials. The HCA content checked by UV spectrophotometric method and HPLC method using C_{16} RP–amide column.

ACKNOWLEDGEMENTS

Two of the authors, (B. Antony and Winny Varghese) would like to express sincere thanks to the UGC for providing grant for conducting the studies on *Garcinia cambogia*.

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