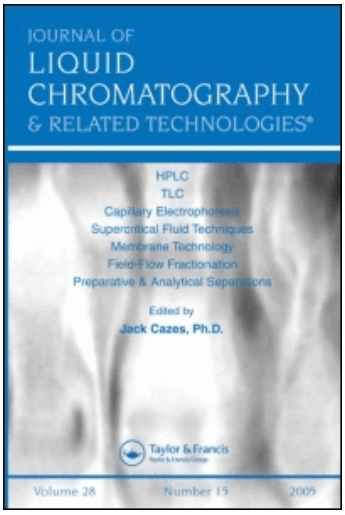

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DETERMINATION OF (-) HYDROXYCITRIC ACID IN COMMERCIAL SAMPLES OF *GARCINIA CAMBOGIA* EXTRACT BY LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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ABSTRACT

A high performance liquid chromatography method was developed for the determination of organic acids in commercial samples of *Garcinia cambogia* extracts. The major organic acid in commercially available extracts of *Garcinia cambogia* has been found to be (-) hydroxycitric acid present to the extent of 51-55%. Tartaric, citric, and malic acids are present in small quantities and have been detected by using 6 m*M* sulfuric acid as the mobile phase with a flow rate of 1.0 mL/min and UV detection at 210 nm.

This method is shown to estimate accurately (-) hydroxycitric acid in the samples of *Garcinia cambogia* extracts. Thus, the method is ideally suited for rapid routine analysis of commercial *Garcinia cambogia* extracts.

INTRODUCTION

(-) Hydroxycitric acid (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) has been found to be the principal acid in the fruits of *Garcinia cambogia*.[1]

Commercially available extracts of *Garcinia cambogia* contain (-) hydroxycitric acid as a calcium salt (Figure 1). It has been shown that (-) hydroxycitric acid (HCA) and its derivatives are potent metabolic regulators of obesity and lipid abnormalities in mammalian systems.[2-6] The antiobesity potency of HCA has been clinically screened and confirmed. HCA as calcium salts are available in the market. Citrimax (TM), HCA-500,[7] GTF, Lipatrol, and Lapodex-2 are some of the commercially available products containing extracts of *Garcinia cambogia* at different levels.[8] Garcinia oral spray formulated with sodium hydroxycitrate.[9] The existing method of assay of HCA in commercial samples of *Garcinia cambogia* extract consists of deionising the sample with cation exchanger and titration of the free acid liberated. This method has the limitation of interference by other organic acids present in the samples. There is no standardized HPLC method for the determination of HCA in commercially available extracts of *Garcinia cambogia*.[10]

In the present study, a simple, specific and reproducible HPLC method for the routine analysis of HCA in commercially available extracts of *Garcinia cambogia* is described.

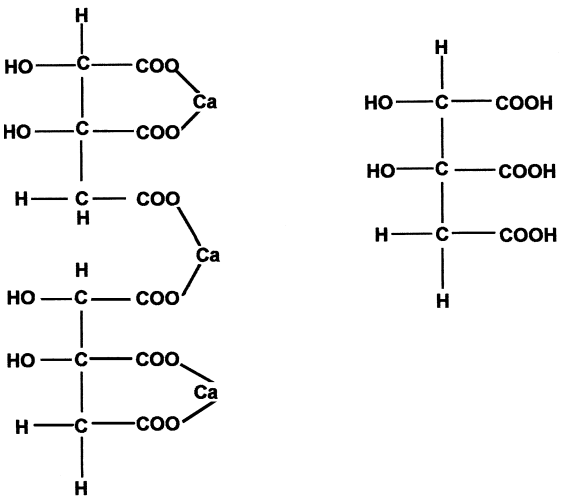

Figure 1. Structure of calcium hydroxycitrate and (-)-hydroxycitric acid.

EXPERIMENTAL

Chemicals

Commercial *Garcinia cambogia* extract samples used were received for the analysis of HCA content from various industries. All solvents used were of HPLC grade and obtained from Merck (Bombay, India). Dowex 50WX8, mesh size 100-200 was obtained from Sigma (St.Louis, MO, USA).

Equipment

A Millipore Swinnex type filter (pore size 0.45 μm) was obtained from Millipore (Bangalore, India). UV spectra measurements were done using Genesys-5 UV-visible spectrophotometer (Milton Roy, NY, USA). The optical rotation of the sample was recorded using Perkin Elmer 243 Polarimeter at 589 nm with 0.5 mL sample solution at 20°C. The chromatographic system consisted of a Shimadzu LC-6A model (Shimadzu, Tokyo, Japan), fitted with a Waters μ-Bondapack™ (Waters Corporation, Milford, MA, USA) C_{18} column (300 × 3.9 mm I.D.) and a System Controller SCL-6A. The injection system used was a 20 μL sample loop. Detection was done by a UV-visible Spectrophotometer SPD- 6AV set at a sensitivity of 0.04 AUFS and a wavelength of 210 nm. Elution was carried out at a flow rate of 1.0 mL/min under isocratic condition.

The compounds were quantified using a Shimadzu C-R4A Chromatopak data processor at chart speed of 2.5 mm/min. IR spectra was recorded on a Bruker-IFS 25 spectrometer using KBr discs. ^{1}H NMR spectra (D_2O) were recorded at 400 MHz on a Bruker AMX 400 FT instrument (Bruker, Rheinstetten, Germany). TMS was used as the internal standard.

HPLC Mobile Phase

6 m*M* Sulfuric acid prepared from reagent-grade sulfuric acid and double distilled water and filtered through 0.45 μm filter membrane.

Preparation of Pure HCA

Standard HCA lactone or *Garcinia cambogia* extract is not commercially available in market. Pure HCA was isolated from the fruits of *Gacinia cambogia* by the method reported earlier and its purity was analyzed by GLC and optical rotation.[11] HCA lactone had a melting point 182°C and optical rotation $[\alpha]^{20}_{D}$ + 99.8°C (c = 1.0; H_2O). These values matched well with those of pure HCA lactone reported values.[11-12] The structure of HCA lactone was further

established by IR and ^{1}H NMR spectroscopy. HCA lactone displayed strong IR bands at 3200, 1760, and 1680 cm^{-1}. ^{1}H NMR spectra of HCA lactone showed two protons at γ-carbon which gives a AB quartet at δ 2.53 and δ 2.74 with J = 17.1 Hz and one proton at α-carbon showed a singlet at δ 5.15. Free HCA was prepared from lactone by treating 50 mg of lactone with 15 mL of 0.1M sodium hydroxide and the solution was heated in a water bath for 60 min.[13]

The sodium hydroxycitrate so obtained was passed through Dowex 50 [H^+]. The resin was washed to neutral pH. The washings and the supernatant from the resin were combined, made up to 50 mL, and filtered. Five standard HCA solutions of concentrations ranging from 0.20 mg/ mL to 1.0 mg/mL were prepared.

Sample Preparation

Method 1

0.1 g of *Garcinia cambogia* extract sample was treated with 30 mL of 8 m*M* sulfuric acid, stirred for 15 min with magnetic stirrer; the supernatant was decanted. The residue of *Garcinia cambogia* extract treated once again with 20 mL of 8 m*M* sulfuric acid, stirred for 15 min for complete desalting.

The supernatants were combined and made up to 100 mL, filtered and stored at 4°C until further use.

Method 2

0.5 g of *Garcinia cambogia* extract sample was suspended in 10 mL of water; 5 g of Dowex-50 [H^+] was added and stirred for 20 min with a magnetic stirrer. The supernatant was decanted and the resin was washed to neutral pH.

Washings and the supernatant were combined, made up to 250 mL, filtered, and stored at 4°C until further use. The HCA content of *Garcinia cambogia* extract samples were determined by acid-base titration and HPLC method (Table 1).

HCA Calibration

10 μL of each of the five working standard solutions containing 2-10 μg of pure HCA was injected onto the HPLC, and elution was carried out as discussed above and peak area responses were obtained. A standard graph for HCA was prepared by plotting the concentration of HCA versus peak area (average of 3 runs).

Table 1

HCA Content of Commercial Samples of *Garcinia cambogia* Extract[a]

Samples	HPLC Method Mean[b] ± SD	Acid-Base Titration Mean[b] ± SD
1	54.74 ± 0.06	61.37 ± 0.45
2	50.73 ± 0.08	57.07 ± 0.35
3	51.89 ± 0.12	57.28 ± 0.83
4	53.90 ± 0.12	59.35 ± 0.83

[a] Samples were prepared as per 2nd Method. [b] Average % recoveries for 5 determinations.

Quantification of HCA in Samples

A known volume of (10-20 μL) of the samples prepared as above was injected into the HPLC and the concentration of HCA was obtained directly from the peak area and by application of the dilution factor. The HCA concentration of the sample was expressed as g/100 g of sample.

Recovery of HCA

The efficiency of HCA recoveries were determined by the standard HCA addition method. Four different spiking levels of free HCA in liquid form were added to 1.0 g of commercial *Garcinia cambogia* extract equivalent to 0.5474 g of HCA. Sample preparations were done by both methods and analyzed as above by HPLC (Table 2).

RESULTS AND DISCUSSION

The existing method for the determination of HCA content in *Garcinia cambogia* extract uses acid-base titration which gives the total acidity of extracts. Generally, GC estimation involves the conversion of acid to volatile silyl derivative. For silylation the sample should be dried completely and the HCA has the tendency for lactonization during drying.[12] Secondly, due to the highly hygroscopic nature of HCA it is rather difficult to dry the sample completely.

In HPLC dilute extracts can be quantified without concentration, drying, and derivatization. HPLC analysis was carried out using different mobile sol-

Table 2

Recovery Study of HCA Added to Selected Commercial Samples of *Garcinia cambogia* Extract

HCA Content in Extracts (g)	Pure HCA Added (g)	1st Method* HCA Recovered (g)	1st Method* Recovery (%)	1st Method* CV (%)	2nd Method* HCA Recovered (g)	2nd Method* Recovery (%)	2nd Method* CV (%)
0.5474	0.25	0.7968	99.93	0.25	0.7919	99.31	0.28
0.5474	0.30	0.8453	99.75	0.38	0.8340	98.42	0.25
0.5474	0.35	0.9019	100.50	0.28	0.8876	98.91	0.28
0.5474	0.40	0.9384	99.05	0.63	0.9461	99.86	0.25

* Average % recoveries for 5 determinations. CV: Coefficient of variation.

vents i.e., 6 m*M* H_2SO_4 , 8 m*M* H_2SO_4, 10 m*M* H_2SO_4 , 12 m*M* H_2SO_4. We found that 6 m*M* H_2SO_4 is the best solvent for the separation of all peaks from commercial *Garcinia cambogia* extract samples (Figure 2).

Table 1 gives the HCA content of four different commercial samples of *Garcinia cambogia* extracts as determined by HPLC and acid-base titration methods. It can be seen that the acid-base titration method gives a slightly higher value for HCA, due to other acids present. The values obtained by the HPLC method accounted only for HCA, since the values correspond to the areas of the HCA peak.

The major organic acid found in *Garcinia cambogia* extracts by HPLC is HCA as shown in the HPLC chromatogram in Figure 2. Three minor peaks are also observed and were identified as tartaric acid, malic acid, and citric acid by co-injection of standard acids. HCA was resolved as a single peak in all samples analyzed with no interference from other compounds. These results indicated that the method is specific for HCA. The identity of the HCA peak was confirmed by determination of relative retention time and by spiking with standard HCA. The retention times of the tartaric acid, HCA, citric acid, and malic acid in all samples were found to be 3.00, 3.78, 5.17, and 6.46 min respectively.

The data in Table 2 shows that almost complete recovery of HCA has been achieved in the recovery experiments. There is an excellent agreement between Method 1 and Method 2 of sample preparation in terms of recovery values. The recovery is given by the ratio of mean peak area obtained by direct injection of a given standard HCA and injection of commercial *Garcinia cambogia* extracts

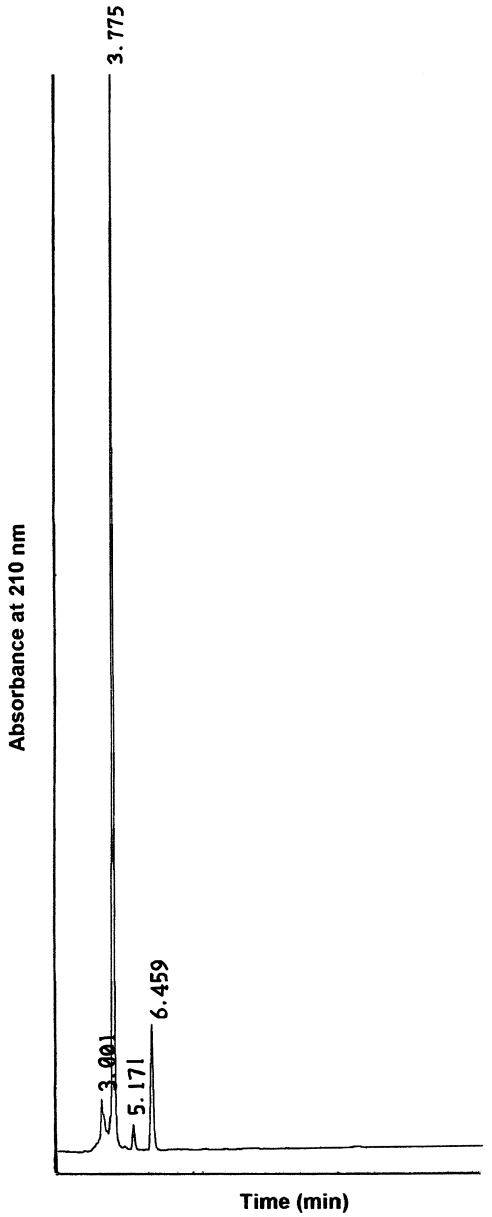

Figure 2. HPLC separation of commercial *Garcinia cambogia* extract.

containing same concentration of standard HCA. The mean recovery of the HCA from extracts of *Garcinia cambogia* were found to be 98.42 to 100.50%, reflecting the reliability and accuracy of the method and it is high enough to quantified. The coefficients of variation resulting from 5 determinations were 0.25 to 0.63% indicating the precision of the method. This method has good reproducibility and accuracy at concentrations ranging from 2- 10 μg of HCA.

CONCLUSION

The present method described is rapid and much more reliable compared to acid-base titration. Values found by the two methods were comparable but were 5-7% higher than the total acid by HPLC.

The coefficients of variation were 0.25 to 0.28%, indicating that the precision of the second method sample preparation is better than the first method.

The method described is suitable for the routine analysis of a large number of commercial samples of *Garcinia cambogia* extract.

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