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Journal of Chromatography A, 806 (1998) 337–339

JOURNAL OF CHROMATOGRAPHY A

Short communication

Determination of organic acids in *Garcinia cambogia* (Desr.) by high-performance liquid chromatography

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Received 3 October 1997; received in revised form 10 December 1997; accepted 14 January 1998

Abstract

The major organic acid in *Garcinia cambogia* (Malabar tamarind) has been found to be (−)-hydroxycitric acid, present in concentrations of 16–18%, using high-performance liquid chromatography with 10 m*M* sulfuric acid as eluent. Citric and malic acids are present in Malabar tamarind in minor quantities. © 1998 Elsevier Science B.V.

Keywords: *Garcinia cambogia*; Hydroxycitric acid; Citric acid; Malic acid

1. Introduction

The principal acid of *Garcinia cambogia* has been found to be (−)-hydroxycitric acid (HCA; 1,2-dihydroxypropane-1,2,3-tricarboxylic acid; Fig. 1). The other three isomers of this compound have not been reported in this material [1]. (−)-HCA is susceptible to lactonisation especially during evaporation and concentration [2]. Recently, it has been shown that HCA is a potent metabolic regulator of obesity and lipid abnormalities in mammalian systems [3–5]. The antiobesity potency of HCA has been clinically screened and confirmed [2]. The existing method of assay of HCA in the fruit of *Garcinia cambogia* consists of titration of fruit extract against standard sodium hydroxide. This method has the limitation of interference by other organic acids present in the samples. A gas chromatographic (GC) method has been reported by Lowenstein and Brunengraber, which involves derivatization of the acid before analysis [6]. In the present study, a simple and versatile high-performance liquid chromatographic (HPLC) method for the determination of HCA is described.

2. Materials and methods

Rinds of *Garcinia cambogia* were obtained from local markets. All reagents/solvents used were of AR/HPLC grade. Dowex 50WX8, mesh size 100–200 was obtained from Sigma (St. Louis, MO, USA). UV spectra were measured using a Genesys-5 UV–visible spectrophotometer (Milton Roy, NY, USA). The chromatographic system consists of a Shimadzu LC-6A model (Shimadzu, Tokyo, Japan) and a multiwavelength detector which was set at 214 nm. HPLC analysis was carried out using a Waters μ-Bondapack^{TM} (Waters, Milford, MA, USA) C_{18} column (300×3.9 mm I.D.). The elution with 10 m*M* sulfuric acid was carried out at a flow-rate of 0.7 ml/min under isocratic conditions. The compounds

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PII S0021-9673(98)00054-5

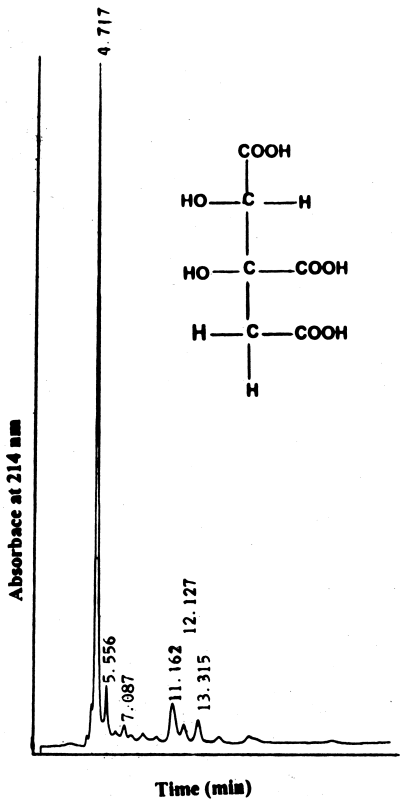

Fig. 1. HPLC-UV chromatogram of Malabar tamarind extract; (−)-hydroxycitric acid.

were quantified using a Shimadzu C-R4A Chromatopak data processor.

2.1. Preparation of pure HCA

Pure (−)-HCA was prepared as lactone in our laboratory according to the method of Singh et al. and its purity was analyzed by GC, acid–base titration and optical rotation [7]. Free HCA was prepared by treating 100 mg of lactone with 0.1 *M* sodium hydroxide and by passing sodium hydroxycitrate through Dowex 50 [H^+]. The resin was washed to neutral pH. The washings were combined and made up to a definite volume and filtered.

2.2. Sample preparation

A 25 g mass of *Garcinia cambogia* rinds was autoclaved at 15 lbs/inch^2 pressure with 50 ml of water for 20 min and filtered. Autoclaving and filtration was repeated twice for complete extraction of the acid. The dark brown extract was decolorised using activated charcoal and filtered. The decolorised extract was concentrated to 25 ml in vacuo and was treated with 50 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 17–19% (w/w) with respect to weight of rinds as determined by acid–base titration using phenolphthalein indicator.

3. Results and discussion

The existing methods for the determination of HCA use an acid–base titration which gives the total acidity of fruit extracts. Determination by means of GC involves the conversion of the acids to volatile silyl derivatives. For silylation the sample should be dried completely and the HCA has the tendency to undergo cyclization to the γ-lactone during drying [2,8]. Secondly, due to the highly hygroscopic nature of HCA it is rather difficult to dry the sample completely. In contrast, in HPLC dilute extracts can be quantified without concentration, drying and derivatization.

HCA showed an absorption maximum at 210–214 nm. Hence 214 nm was used for the HPLC detection. The major organic acid found in Malabar tamarind fruits by HPLC is HCA as shown in the HPLC chromatogram in Fig. 1. Four minor peaks are also observed of which two were identified as citric and malic acids by coinjection of standard acids. The other two minor peaks are unidentified. Table 1 gives the HCA content as determined by the HPLC method

Table 1
HCA content in three samples of Malabar tamarind determined by HPLC and the acid–base titration method

Samples	HPLC method (g/100 g)		Acid–base titration (g/100 g)	
	Mean[a]	R.S.D. (%)	Mean[a]	R.S.D. (%)
I	16.0	6	17.0	16
II	18.0	6	19.2	14
III	17.2	4	18.0	15

[a] $n=6$.

and the acid–base titration method in three different samples of Malabar tamarind. As expected, the acid–base titration gives slightly higher values for HCA, due to the other acids present. The high relative standard deviation (R.S.D.) value in the case of acid–base titration (Table 1) may be due to a lesser sensitivity of the end point compared to HPLC detection and interference of other acids present in the extracts.

HCA was resolved as single peak in all samples analyzed with no interference from other compounds. These results indicated that the method is sufficiently selective. The identity of the HCA peak was confirmed by determination of relative retention time and by spiking with standard HCA. The relative retention times of the HCA, citric acid and malic acid in all samples were found to be 4.72, 5.56 and 7.09 min respectively. The percent recovery of each sample was calculated by the ratio of mean peak-height obtained from direct injection of standard HCA and injection of fruit extracts containing the same volume of standard HCA. Also, there was no considerable difference in the recovery rates at different concentrations of standard HCA. The mean recoveries of the HCA from extracts were found to be 98±3% and this is high enough to be quantified. This method, validated for concentrations ranging from 2–10 μg/ml has a good reproducibility and accuracy.

In conclusion, the present method is simple, and sufficiently selective and accurate for the determination of HCA in commercial samples of *Garcinia cambogia*.

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