
A HPLC Determination of Betacarotene in the Fruit Juice of *Passiflora edulis* Sims

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A simple, accurate and reproducible HPLC method for the determination of betacarotene in the ripe fruit juice of *Passiflora edulis* Sims was developed. The method involves separation of betacarotene using an isocratic mobile phase consisting of acetonitrile, methylene chloride and methanol in the ratio of 82:12:16 at 436 nm using photo diode assay detector. The sensitivity was found to be 0.25 mg. The betacarotene content of 3.21%w/v was observed in test sample. The calibration curve was linear in the range of 0.25 mg to 2.50 mg with correlation co-efficient of 0.987998 indicating good linearity between concentration and area. The method provides good resolution and separation of betacarotene from other constituents of total carotenoids. An average recovery of 99.08% w/w shows the reliability and suitability of the method.

The edible Passion fruit (*Passiflora edulis* Sims) originated in Brazil is cultivated throughout the tropics and subtropics, where it is a fresh fruit crop important in local diets and is available in most markets¹. The juice from the ripe fruit of *Passiflora edulis* Sims is highly nutritious, contains ascorbic acid and is rich in carotene and acid content². The plant is used as antispasmodic, narcotic, sedative, anthelmintic, blood pressure reducer, expectorant and the fruits are a good source of vitamin C³. The pulp of the fruit is used as a stimulant and tonic^{4,5}.

Betacarotene is a natural food substance found in red, yellow, and orange fruits and vegetables⁶. Carotenes have been implicated as anticancer compounds in numerous compounds in numerous studies⁷. Since betacarotene is the main active principle of the ripe fruit juice of *Passiflora edulis* Sims^{8,9}, estimation of it is essential for standardization of the fruits and its various herbal formulations. Hence, an attempt has been made to develop a precise, sensitive and reproducible method for quantitative estimation of betacarotene in the total carotenoids from the juice of the ripe fruits of *Passiflora edulis* Sims with reference to standard betacarotene.

The fruits of *Passiflora edulis* Sims were obtained in full ripe state from the inner parts of Kerala (Kottayam) identified by the Botanical Survey of India, Coimbatore and were

stored at 4° until analyzed. An isocratic HPLC system (Shimadzu) consisting of LC-10AT liquid pump, SPD-10A UV/Vis detector, ODS C-18 RP-column (4.6 mm I.Dx25 cm), 25 µl Hamilton injecting syringe and window based single channel software was used.

Betacarotene reference standard (1 mg/ml, Hi-media Labs, Mumbai) was prepared in HPLC grade methylenechloride solution. It was further diluted to yield final concentrations of 12.5, 25, 50, 75 and 125 µg/ml.

A total of 600 g of passion fruit pulp was homogenized with 1000 ml of distilled water for 30 s. The homogenate was cleared by filtration through a muslin cloth in a Hafico tincture press at 400 atm. This clarified juice, possessing a pH value of 3.0, was stored at 4° overnight. The juice was further concentrated by vacuum distillation below 40°. The total carotenoids were extracted from the dried juice by macerating with 95% ethanol and further treated with methylene chloride and 60% potassium hydroxide. Light petroleum solution was added and kept overnight at -10° to precipitate the steroids. The extraction was repeated six times using 10 ml methylene chloride; filtrates were pooled, transferred to 100ml volumetric flask and made up to the volume. This solution was used for HPLC analysis.

Five different concentrations of standard betacarotene (12.5, 25, 50, 75 and 125 µg/ml) were injected using 25 µl Hamilton injecting syringe. The mobile phase was acetonitrile:methylene chloride:methanol (82:12:16) at 436 nm. The chromatogram was scanned up to 20 min. The chro-

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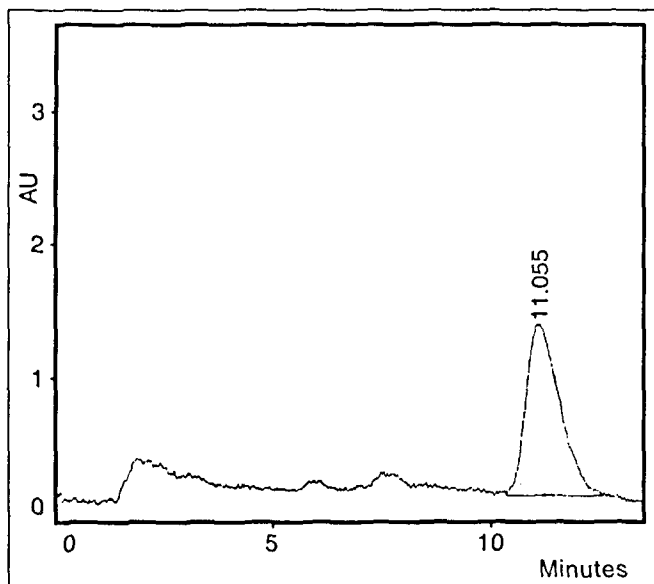


Fig 1: HPLC chromatogram of standard betacarotene
 Chromatogram showing the peak of standard betacarotene at 11.055 min using the mobile phase acetonitrile:methylene chloride:methanol (82:12:16) and scanned at 436 nm

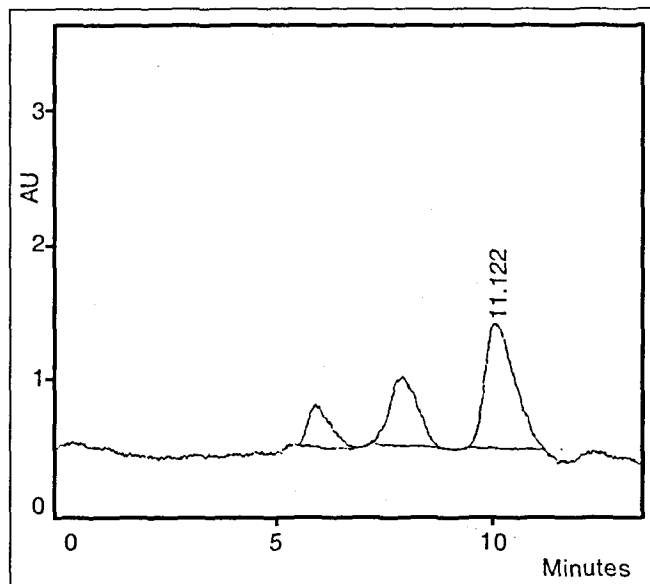


Fig 2: HPLC chromatogram of betacarotene extracted from the juice of ripe fruits of *Passiflora edulis* Sims
 Chromatogram of the total carotenoids from the ripe fruit juice of *Passiflora edulis* Sims shows three peaks. The peak resolved at 11.122 min indicates betacarotene when compared to the HPLC chromatogram of standard

matogram of standard betacarotene and sample are shown in fig.1 and 2, respectively. The amount of betacarotene in test sample was determined from the linear regression equation of calibration graph plotted between concentration and area. The correlation coefficient was found to be 0.987998.

A varying amount of standard betacarotene (10, 20 and 50 μg) was added to about 1 mg of total carotenoid in which the contents of betacarotene was estimated previously by proposed method. The samples were analyzed separately and the contents of betacarotene were quantified using proposed method and percent recovery calculated.

The proposed method is a simple, precise and sensitive method for estimation of betacarotene in the total carotenoids from the ripe fruit juice of *Passiflora edulis* Sims. The retention time of betacarotene was about 11 min. The chromatograms of standard betacarotene and test sample are shown in fig.1 and 2, respectively. The average betacarotene content in test sample was found to be 3.21% w/v. The calibration coefficient was 0.987998 indicating good linearity between concentration and area. The method provides for good resolution and separation of betacarotene. Table 1 indicates an average recovery of 99.08%w/w, which shows reliability, and suitability of the method. Hence this

TABLE 1: METHOD VALIDATION AND RECOVERY OF BETACAROTENE FROM *PASSIFLORA EDULIS* SIMS

Sample amount (A) (μg)	Amount of betacarotene present (B) (μg)	Amount of betacarotene added (C) (μg)	Total betacarotene (B+C) (μg)	Total betacarotene found (μg)	% recovery E/Dx100
1010	31.41	10.0	41.41	41.38	99.93 \pm 0.25
1000	31.20	20.0	51.20	50.13	97.91 \pm 0.38
1020	31.62	50.0	81.62	81.15	99.42 \pm 0.43

Sample taken is the total carotenoids fraction from ripe fruit juice of *Passiflora edulis* Sims. Average percent recoveries of betacarotene from the total carotenoids of *Passiflora edulis* fruit juice is 99.09 \pm 0.20%. The values are average of three determinations, \pm standard deviation.

method can be easily used for the standardization of the active constituent betacarotene in the ripe fruits of *Passiflora edulis* Sims and its marketed antioxidant formulations.

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