

HPLC-MONOLITHIC COLUMN FOR THE DETERMINATION OF RETINOL AND ALPHA-TOCOPHEROL IN AVOCADO OIL

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Abstract: A rapid high performance liquid chromatographic procedure for the determination of retinol and α -tocopherol from avocado oil was proposed. Two difference extraction methods were investigated to extract oil from avocado sample. This method was developed and validated for various chromatographic conditions for simultaneous determination of retinol and α -tocopherol in avocado oil samples. The oil sample was analyzed on Chromolith C-18 (4.6 mm $\times$ 25 mm), using methanol and water as mobile phase with gradient elution system. The analytes were detected at 292 and 325 nm, respectively by spectrophotometry. The flow rate was used at 2 mL min⁻¹ to complete separation of both analytes and was achieved in 6 minute. Under the optimum conditions, retinol and α -tocopherol could be determined within a concentration range of 1-40 μ g mL⁻¹ which can be expressed by the regression equation $y = 32222x + 5107$ ($r^2 = 0.9990$) and $y = 5742x + 3400$ ($r^2 = 0.9990$), respectively. The limit of detection and quantitation were found to be 0.044 μ g mL⁻¹ and 0.120 μ g mL⁻¹ for retinol and 0.134 μ g mL⁻¹ and 0.364 μ g mL⁻¹ for α -tocopherol respectively. The proposed method was applied to the determination of retinol and α -tocopherol contents in avocado oil samples. The retinol and α -tocopherol contents were found to be 0.002-0.036 mg g⁻¹ and 0.033-0.142 mg g⁻¹ respectively. The development method is simple, rapid and especially suitable for analysis of retinol and α -tocopherol in avocado oil samples.

1. Introduction

Avocado (*Persea americana* Miller) is an important commercial tropical fruit. Botanically, avocados can be classified into four strains: West Indian, Guatemalan, West Indian-Guatemalan hybrid, and Mexican [1-2]. In Thailand, avocado plants were introduced by missionaries and were planted initially in Nan province in northern Thailand about 90 years ago. Some seedlings from these introductions are currently found around this area. The introduced cultivars were Buccanaer, Booth 7, Booth 8, Catalina, Choquette, Hall, Lula, Peterson, Queen, Taylor and Waldin. The Hass, Bacon and Reed cultivars were introduced also from California in 1993. The harvesting time of avocado cultivars in Thailand extends from July to December. Harvesting time of the same cultivar grown under different climatic conditions may vary from one to three weeks. The introducing avocado cultivars into Thailand were to evaluate their suitability under local conditions. Due to their high nutritive value and wide utilization

possibilities, avocado fruits were becoming more popular in Thailand. Apart from being consumed as fresh fruits for their palatability, food value with high protein, vitamin E and digestible fat content, their oil is used also in the cosmetic industry [3-4].

Avocado is one of the finest salad fruits. It is a good source of vitamins (A, B, C, E, folacin, niacin etc.) and minerals (iron, magnesium, potassium etc.). The studies on avocados showed that they contained potentially anti-carcinogenic lipophilic components such as carotenoids [5]. The lipophilic extract of avocado inhibited prostate cancer cell growth [6], induced apoptosis in human breast cancer cells [7], and suppressed liver injury [8].

To determine avocado oil different analytical procedure; thin layer chromatography [9] and high performance liquid chromatography [10-14] are utilized. No articles on HPLC analysis combined of retinol and α -tocopherol from avocado oil were reported in the literature.

The present paper, the HPLC-monolithic silica rod column was developed for simple, rapid and reliable HPLC method with spectrophotometric detection for the quantitation of retinol and α -tocopherol in avocado oils. This method has been applied to the development of the HPLC method using monolithic silica rod column technology and their applications to the quantitation of retinol and α -tocopherol in avocado oils (Hass, Booth 7 and Buccanaer) collected from Phetchabun and Tak province.

2. Experimental

2.1 Apparatus and instruments

The chromatographic system for the separation and analysis of retinol and α -tocopherol in avocado oils were carried out with Shimadzu Model SCL-10A liquid chromatograph, thermostatic column compartment, online degasser and an UV-visible detector model SPD-10A. The analytical column used was 25 $\times$ 4.6 mm Chromolith® Flash RP-18e column (Merck KGaA 64271 Darmstadt, Germany). Mobile phase was a mixture of various ratios of methanol and water with gradient elution system. The flow rate was adjusted to 2 mL min⁻¹. The injection volume was adjusted to 20 μ L and the UV-visible absorptions wavelengths for detection were 292 and 325 nm. The sample solution was prepared and vacuum-filtered through 0.45 μ m nylon membrane (USA) before use.