Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 788-790

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Quantification of β-Carotene from *Diplocyclos palmatus* Jeff. Fruits Rind by Using High Performance Thin Layer Chromatography

AVINASH PATIL*, DARSHANA A. PATIL, ABHISHEK SHARMA and NARESH CHANDRA

Department of Botany-Herbal Sciences, Birla College, Kalyan-421 304, India

*Corresponding author: E-mail: dravinashpatil@rediffmail.com

(*Received*: 16 April 2010; *Accepted*: 5 October 2010) AJC-9163

A simple TLC method has been developed for the quantification of β-carotene from fruit rind of *Diplocyclos palmatus* Jeff., using HPTLC plate pre-coated with silica gel 60 F_{254}. The method was developed in petroleum ether (8 mL) and validated in terms of precision, repeatability and accuracy. The linearity range was found to be 6-60 μg mL^{-1} with correlation coefficients of 0.999, which is indicative of good linear dependence of peak area on concentration. The method permits reliable quantification and showed good resolution and separation from other constituents of extract. Accuracy of the method was checked by conducting recovery studies at three different levels and the average percentage recoveries were found to be 99.41 ± 0.32 %. The amount of β-carotene in fruit rind of *Diplocyclos palmatus* Jeff. was found to be 0.0065 ± 0.0003 mg g^{-1}. The proposed method was found to be simple, precise, specific, sensitive and accurate. It can be used for routine quality control of herbal material containing β-carotene.

Key Words: HPTLC, Quantification, *Diplocyclos palmatus* Jeff., Fruit rind.

INTRODUCTION

β-Carotene (Fig. 1) is probably the best known of the group of carotenoids occurring in fruits and vegetables. Of the 50 or so carotenoids included in the human diet, β-carotene is the most common, followed by α-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene. These carotenoids have two different functions in nature: their colour makes flowers and animals attractive and as bioactive substances, they support essential processes in the metabolism of micro-organisms, plants, animals and humans[1].

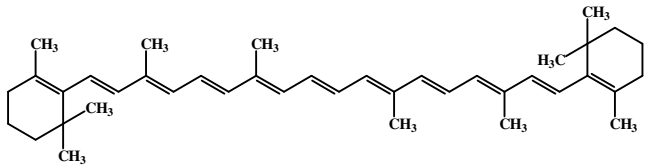


Fig. 1. Structure of β-carotene

β-Carotene is a powerful antioxidant and observational and prospective epidemiological studies have shown an inverse relationship between degenerative diseases linked to oxidative stress and carotenoid intake and/or blood levels. It appears that β-carotene can promote health when taken in sufficient quantities, but may have adverse effects when taken in large doses by subjects who are long-term heavy smokers or who have been exposed to asbestos[2]. β-Carotene is the most available and important source of pro-vitamin A in the diet of most people living in developing countries, providing about 66 % of vitamin A in their diets[3].

Diplocyclos palmatus Jeff., family: Cucurbitaceae, (Syn. *Bryonopsis laciniosa*), found throughout India, known as Lingini, Shivlingi in Ayurveda. Plant is used in venereal diseases, seeds are used against inflammatory, vaginal dysfunction and as a fertility promoting drug. Powdered seeds with root are given to help conception in women[4]. Leaves of *Diplocyclos palmatus* Jeff. shows analgesic and antipyretic activity[5]. Whole plant is used to treat adenopathy, ague, asthma, bronchitis, carbuncles, cholera, colic, consumption, convulsions, cough, delirium, fertility, headache, megalospleny, paralysis, phthisis and snake bite. The chloroform extract of *Diplocyclos palmatus* Jeff., has exhibited significant antiinflammatory activity[6]. Analgesic and antipyretic activity of methanol extract of *Diplocyclos palmatus* Jeff., also has been shown in standard animal models[5].

Several HPLC methods have been reported for the quantification of β-carotene from different sources. Park *et al.*[7] reported a method for the isolation of carotenoid pigments from yeast cells using HPLC for isolation of carotenoid pigment and TLC for separation of β-carotene using toluene:acetone (7:3, v/v) at R_f 0.90. In the present research work petroleum ether (8 mL) as mobile phase, gave a proper

separation of β-carotene at R_f 0.30. Therefore, the present method can be used for quality control and quantitation of β-carotene from other biological sources easily.

EXPERIMENTAL

Fruits of *Diplocyclos palmatus* Jeff. were collected from Kalyan, Maharashtra, India in January 2010. The fruits were authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. The collected fruits were separated from seeds and rind was used for the estimation of β-carotene.

All the chemicals used in the experiments were of analytical grade. Reference standard β-carotene (purity 98 %, w/w) was purchased from HiMedia Pvt. Ltd., Mumbai, India.

Sample preparation: Fresh fruit rind of *Diplocyclos palmatus* Jeff. (1 g) were vortexed extracted with acetone (20 mL) for 10 min. The extract was filtered and subjected to TLC for estimation of β-carotene.

Preparation of standard solution: The stock solution of β-carotene (10 mg mL^{-1}) was prepared in acetone. The stock solution was quantitatively transferred into a 10 mL volumetric flask to give solution of appropriate concentration range of 6-60 μg mL^{-1} and made to volume with acetone. Standard solutions were prepared by dilution of the stock solution.

Chromatographic conditions: Chromatography was performed on aluminium HPTLC plates coated with silica gel 60 F_{254} (Merck # 5554). Before use, plates were pre washed with methanol and dried in an oven at 105 ºC. Samples (10 μL) were spotted as 15 mm, starting 14 mm from edge of the plates, by means of a Camag Linomat V sample applicator. The plates were developed up to a distance of 90 mm from the base of plate in Camag twin-trough chamber previously equilibrated with mobile phase for 20 min. The mobile phase consisted of petroleum ether (8 mL). The chromatographic conditions had been previously optimized to achieve the best resolution and peak shape. After development, plates were dried under air current at room temperature and densitometric evaluation of the plates was performed at 450 nm (λ_{max}) in reflectance-absorbance mode using tungsten lamp with a Camag Scanner 3.

The identity of the band of β-carotene in the sample was confirmed by overlaying the chromatogram of sample with that of the β-carotene and by comparing their R_f (0.30). The chromatographic plate of standard β-carotene and *Diplocyclos palmatus* Jeff. samples is shown in Fig. 1.

Linearity: The linearity of detector response was evaluated in triplicate using seven different concentrations ranging from 6-60 μg mL^{-1} of β-carotene. The plot was linear in the range 6-60 μg mL^{-1} (Table-1).

Method validation: The method was validated for precision, repeatability accuracy. Precision of the method was checked by repeated scanning (n = 5) of the same spot of β- carotene seven times. The repeatability of sample application and measurement of peak area were expressed in terms of % COV. Variability of the method was studied by intra-day precision and inter-day precision. In order to estimate limit of detection (LOD) and limit of quantitation (LOQ), blank acetone was spotted to determine signal to noise ratio. LOD was considered as 3:1 and LOQ as 10:1. Accuracy of the method was tested by performing recovery studies at three different levels (0, 50 and100 % addition)[8].

TABLE-1
METHOD VALIDATION PARAMETER FOR THE QUANTITATION OF β-CAROTENE

Parameters	β-Carotene
Linearity range (μg mL^{-1})	6-60
Slope (m)*	449.1
Intercept (c)*	1765
Correlation coefficient (R^2)	0.999
LOD (μg mL^{-1})	2
LOQ (μg mL^{-1})	4
Intraday precision (n = 3, % COV)	0.82
Interday precision (n = 3, % COV)	1.14
System Suitability (n = 3, % COV)	0.74

*Of the equation y = mx + c, where y is peak area, m is the slope, x is the concentration and c is the intercept.

RESULTS AND DISCUSSION

In the present study, we quantitate β-carotene from fruit rind of *Diplocyclos palmatus* Jeff. by HPTLC densitometric method using silica gel HPTLC plate. The developed method was validated as per the ICH guidelines (Tables 1-3). It was found that β-carotene resolved well at R_f 0.30 (Fig. 2) in the solvent system of petroleum ether (8 mL). Specificity of the method was carried out by matching spectra of β-carotene with *Diplocyclos palmatus* Jeff. and it was found to be specific at 449 nm (Fig. 4).

TABLE-2
RECOVERY STUDY OF β-CAROTENE FROM FRUIT RIND OF *Diplocyclos palmatus* Jeff.

Marker compounds	Amount present in the sample (mg)	Amount added (mg)	Recovery (%)	Mean recovery ± SD
β-Carotene	0.0066	–	99.21	99.41 ± 0.32
	0.0066	0.0033	99.79	
	0.0066	0.0066	99.24	

*Mean ± SD, n = 3.

TABLE-3
β-CAROTENE QUANTIFIED FROM FRUIT RIND OF *Diplocyclos palmatus* Jeff.

Sample tested	Content of β-carotene (mg g^{-1})
Fruit rind of *Diplocyclos palmatus* Jeff. with β-carotene	0.0065 ± 0.0003

*Mean ± SD, n = 3.

Linearity: A good linearity was achieved in the concentration ranges of 6-60 μg mL^{-1} for β-carotene. The regression equations and correlation coefficient for the reference were y = 499.1x + 1765, R^2 = 0.999 (Table-1).

Instrumental precision and intra-day and inter-day precision: Instrumental precision was checked by repeated scanning of the same spot of β-carotene five times each. Standards of β-carotene were spotted both at intra-day (spotting each concentration three times within 24 h) and inter-day (spotting each concentration three times during 3 days intervals) to check the precision. The results are expressed as % COV.

Recovery: The recovery was used to evaluate the accuracy of the method. The present recovery was calculated. Recovery studies at three different levels were done on *Diplocyclos palmatus* Jeff. by accurately spiked with various concentrations of β-carotene just prior to the extraction. The percentage

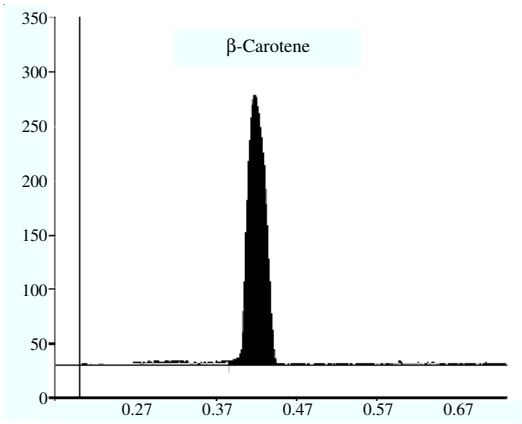
Track 1: Standard β-carotene
Track 2: Acetone extract of *Diplocyclos palmatus* Jeff.

Fig. 2. Chromatogram of *Diplocyclos palmatus* Jeff. with β-carotene

recovery at three different levels for β-carotene was found to be 99.41 ± 0.32 %. The results are shown in Table-2.

Limit of detection and limit of quantitation: The LOD and LOQ were found to be 2 and 4 μg mL^{-1} for β-carotene.

Quantitative determination: Samples were extracted, as described above and analyzed by HPTLC. The content of β-carotene was determined by the corresponding regression equation and results are summarized in Table-3. The result indicated β-carotene was detected in *Diplocyclos palmatus* Jeff. The chromatogram and densitograms of standards along with plant materials are represented in Figs. 2 and 3. Fruit rind of *Diplocyclos palmatus* Jeff. was found to contain 0.0065 ± 0.0003 mg g^{-1} of β-carotene (Table-3).



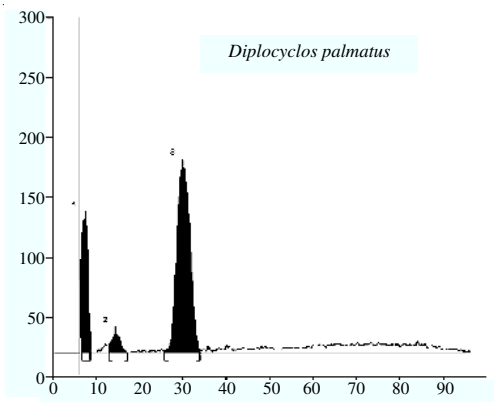


Fig. 3. Densitogram of *Diplocyclos palmatus* Jeff. with β-carotene

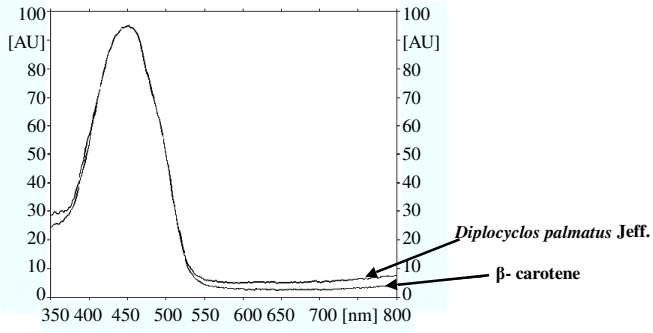


Fig. 4. Spectral analysis of *Diplocyclos palmatus* Jeff. with β-carotene

Conclusion

An improved HPTLC method has been developed for quantitation of β-carotene in fruit rind of *Diplocyclos palmatus* Jeff. The method was found to be simple, precise, accurate, specific and sensitive and can be used for routine quality control of herbal raw materials and for the quantification of β-carotene in plant materials.

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