Original Article

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Analysis of β-Carotene in Carrot by Spectrophotometry

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Abstract

This project aimed to develop a simple UV spectrophotometric method for the analysis of β-carotene in carrot. Extraction of β-carotene from carrot was simply by liquid-liquid extraction and UV absorbance was measured at 461 nm. The developed method was valid for its linearity, accuracy, precision, limit of detection (LOD) and limit of quantitative (LOQ). The UV spectrophotometric method illustrated excellent linearity (r^2 = 0.999) in a range of 1-8 μg/mL. Precision was good with relative standard deviation of less than 6.4% and average recovery was 100.2%. The LOD of UV spectrophotometric measurement was 0.04μg/mL and the LOQ was 0.11μg/mL. The proposed method could be applied to the analysis of β-carotene in carrot samples from different sources. The method is reliable, rapid and inexpensive and could be transferred to quality control laboratories.

Key words: Spectrophotometry, β-carotene, carrot

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INTRODUCTION

The term "carotenoids" refers to a family of more than 600 different plant pigments, which are responsible for many colors (red, orange and yellow etc.) of plant leaves, fruits and flowers, as well as the colors of some birds, insects, fish and crustaceans. Carotenoids, which have polyisoprenoid structures, are generally found in plants, algae, photosynthetic bacteria, non-photosynthetic bacteria, yeasts and molds. One of the most important physiological functions of carotenoids in human nutrition is to act as pro-vitamin A (vitamin A precursors). Pro-vitamin A carotenoids support the maintenance of healthy epithelial cell differentiation, normal reproductive performance, and visual functions[1,2]. Additionally, non pro-vitamin A carotenoids (e.g. lutein, astaxanthin, zeaxanthin and lycopene) also play an important role in human health as biological antioxidants, protecting cells and tissues from the oxidative damaging effects of free radicals and singlet oxygen[1,2]. Many studies show strong correlations between carotenoids intake and a reduced risk of some diseases, such as cancer[3-5], atherogenesis[6,7], bone calcification[8], eye degeneration,[9,10] immune function[11-13] and neuronal damage[14]. Among the carotenoids, β-carotene (Figure 1) is popular to consumers. β-carotene belongs to the carotene class, which is one of the most abundant found in the diet and is used as food colorants.

Different methods have been proposed for the analysis of carotenoids including β-carotene. For example, Raman spectroscopy was used for detection of carotenoids in human skin[15], in the human eye[16], and in liver corpus luteum cells[17]. This method exhibits good detection limits and allows non-invasive analysis. Classical-column chromatography and thin-layer chromatography (TLC) were used for the determination of carotenoids[18-20]. However, these methods are time consuming and require large amounts of samples. In addition, their separation efficiency and reproducibility are poor with low recoveries of the analytes. Therefore, TLC is mainly used for preliminary examinations to give an indication of the number and variety of carotenoids present and to help in the selection of a suitable separation and purification procedure for a given mixture. Among the high performance separation methods, gas chromatography (GC) is not normally used because of low volatility and thermolability of carotenoids. High performance liquid chromatography (HPLC) is commonly used for the determination of carotenoids. There are several reports on the determination of different carotenoids by HPLC with C_{18} or C_{30} reverse phase (RP) column operated with an isocratic or a gradient elution using the mixtures of different organic solvents as mobile phase and different detectors such as UV-Vis, diode array detector (DAD), mass spectrometry (MS), nuclear magnetic resonance (NMR), thermal lens detector and electrochemical detector (ED)[21-29]. Analysis of carotenoids by capillary zone electrophoresis (CZE) is inapplicable because of the absence of charges on the carotenoid molecules. However, capillary electrochromatography (CEC) using a Hypersil ODS packed column has been utilized for the analysis of highly hydrophobic carotenes (*i.e.* β-carotene, lycophene, xanthophylls and lutein) in vegetables[30]. These methods (e.g. Raman spectroscopy, HPLC, CZE) offer high efficiency, but require costly instrument and skillful operators.

The aim of this work is to develop a simple and rapid method for determination of β-carotene in carrot by spectrophotometry. Extraction of β-carotene from carrot was investigated and the proposed method was validated and applied for quantitation of β-carotene in carrot samples obtained from various places. Spectrophotometric method shows potential for the analysis of β-carotene because the pigment can absorb radiation in visible region (400-600 nm). Carrot is widely consumed vegetable, which contains high amounts of β-carotene. However, β-carotene content in carrot can be varied depending on sources. Knowing the exact amounts of β-carotene can benefit consumers and food industry in quality control of carrot originated health products.

MATERIALS AND METHODS

Chemicals and instruments

Tetrahydrofuran (THF) and dichloromethane (DCM) were from Labscan (Bangkok, Thailand). Sodium chloride (NaCl) was from Merck (Darmstadt, Germany), anhydrous sodium carbonate (anh. Na_2CO_3) from APS Chemicals Limited (Bangkok, Thailand) and all – *trans β*-carotene from Sigma (St. Louis, MO, USA).

UV absorption was performed in a range of 200-800 nm on a Shimadzu UV-Vis spectrophotometer model 160A (Kyoto, Japan). Sample solutions were centrifuged by a 6930 KUBOTA centrifuge (STE CO., LTD. (Bangkok, Thailand). Water was deionized (DI) water.

Standard and sample preparations

Standard β-carotene for identification was prepared in DCM to obtain 4 µg/mL. β-carotene in carrot samples was extracted by procedures described by Herrero-Martinez et al[30]. Briefly, carrots were washed with DI water and cut into small pieces. Eighty grams of carrot was blended with 8 g anhydrous sodium carbonate and mixed with a mechanical blender. Ten grams of the mixture was transferred into a centrifuge tube, added with 20 mL THF and mixed for 2 min under cold water. The mixture was centrifuge at 5000 *g* for 5 min and the supernatant was collected. Extraction was performed by adding 15 mL DCM and 15 mL of 10% w/v NaCl into the supernatant and shaken for 2 min. The extraction was repeated twice, organic layer was collected and evaporated under nitrogen steam. The residue was kept at -20 °C, reconstituted with 5 mL DCM and diluted (1/40-fold) with DCM prior UV measurements.

Method validation

Method validation was evaluated in terms of linearity, accuracy, precision, limits of detection (LOD) and quantitation LOQ). Linearity of the method was performed in a range of 2-4 µg/mL, linear regression and correlation coefficient (r^2) were calculated using Microsoft Excel®. Precision was determined from repeatability, intra-day and inter-day precision and relative standard deviation (RDS) was calculated. Repeatability was from repetitive UV measurement of standard β-carotene solution at 3 µg/mL (n=9). Intra-and inter-day precision was determined from UV measurement of standard β-carotene solution at 2, 3 and 4 µg/mL on the same day (n=3) and on different days (n=6). Accuracy was performed by standard addition method and recovery (R) was calculated. Standard β-carotene solution at 2, 3 and 4 µg/mL were added into sample extract and UV absorption of the spiked and unspiked samples was measured (n=3). %R was calculated from (amount found/amount added) x 100. LOD and LOQ were calculated from (3.3 x SD)/s and (10 x SD)/s, where SD and s were standard deviation of blank measurement (n=9) and slope of calibration curve, respectively.

Applications

Carrot samples from 7 different sources were purchased from various markets and supermarkets. They were extracted as described earlier. UV-measurment was performed at 461 nm (n=3). β-carotene contents in carrot samples were calculated based on the calibration curve.

RESULTS AND DISCUSSION

Identification

UV spectrum of β-carotene was scanned from 200 to 800 nm and maximum absorption was obtained at 461 nm. This was in good agreement with that reported in literatures[31,32]. UV spectrum of carrot extract could be superimposed to that of the standard and also showed the maximum UV absorption at the same wavelength (Figure 2). This confirmed that the extraction procedure was valid and the extract contained β-carotene.

Method validation

Initially, linearity of the method was performed in a range of 2-4 µg/mL and the method showed good linearity with a regression of y = 0.1069 x – 0.0057 (r^2 = 0.9981) (Figure 3A and 4A), where x and y

were β-carotene concentration and UV absorption at 461 nm, respectively. Later the linearity range was increased from 1-8 µg/mL since carrot might contain β-carotene in higher amounts. At the higher range, the method also provided acceptable linearity with regression of y = 0.096x -0.002 (r^2 = 0.9990) (Figure 3B and 4B).

Method repeatability showed RSD of 0.25%. Intra-and inter-day precision revealed RSD in ranges of 1.27-3.13% and 5.32-6.44%, respectively (Table 1). Some of these RSD values were slightly high since β-carotene was unstable and easily degraded at room temperature. However, they were in acceptable ranges according to AOAC (24) regulation. Method accuracy showed %R of 96.3-103.0 with RSD of 3.5% (Table 2). LOD and LOQ were 0.04 and 0.11 µg/mL, respectively.

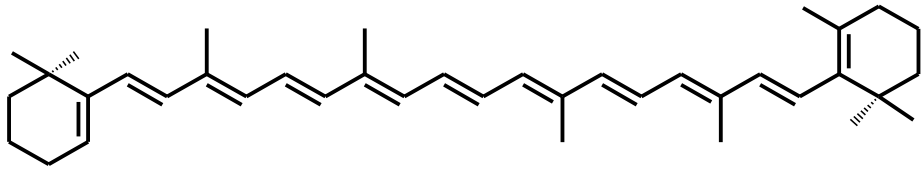
Validation data indicated that the proposed method showed good linearity, precision, accuracy and sensitivity, which could be used for determination of β-carotene in carrot.

Application

The proposed method was applied to the analysis of β-carotene content in carrot samples from different 7 sources. The amount of β-carotene varied from 6.19-14.59 mg/100g carrot with RSDs of 1.02-7.11 (Table 3). UV spectra of β-carotene in carrot samples are shown in Figure 5.

CONCLUSION

A spectrophotometric method was established for the determination of β-carotene in carrot. Extraction of β-carotene from carrot was conveniently performed by liquid extraction by DCM. Method validation confirmed that the method was linear, precise, accurate and sensitive for the estimation of β-carotene in carrot. Sample matrices did not interfere the analysis since no extra spectra or absorbance was observed, which was confirmed by the average recovery about 100.0%. It is highly recommended to freshly prepare the standard and extract prior UV measurements to prevent degradation of β-carotene and to improve the method precision.The method could be applied to identification and quantitation of β-carotene in carrot. Varied amounts of β-carotene in carrot samples were due to several environmental factors (e.g. nutrient, water, etc.), age and species of carrots. Nevertheless the amounts found (6.19-14.59 mg/100 g carrot) were similar to those reported by Herrero-Martinez et al (1.8-14.7 mg/100 g carrot)[30,33,34]. Comparing the other methods (e.g. HPLC and CE), the proposed method was rapid, simple and inexpensive. Moreover, the method could be adopted by most quality control laboratories of food and drug industries since a spectrophotometer is a common instrument, which is generally available.



β-carotene

Figure 1. Structure of β-carotene.

Table 1. Precision data

Concentration (µg/mL)	%RSD		
	Repeatability	Intra-day	Inter-day
2.0	-	3.13	6.44
3.0	0.25	1.75	5.32
4.0	-	1.27	5.52

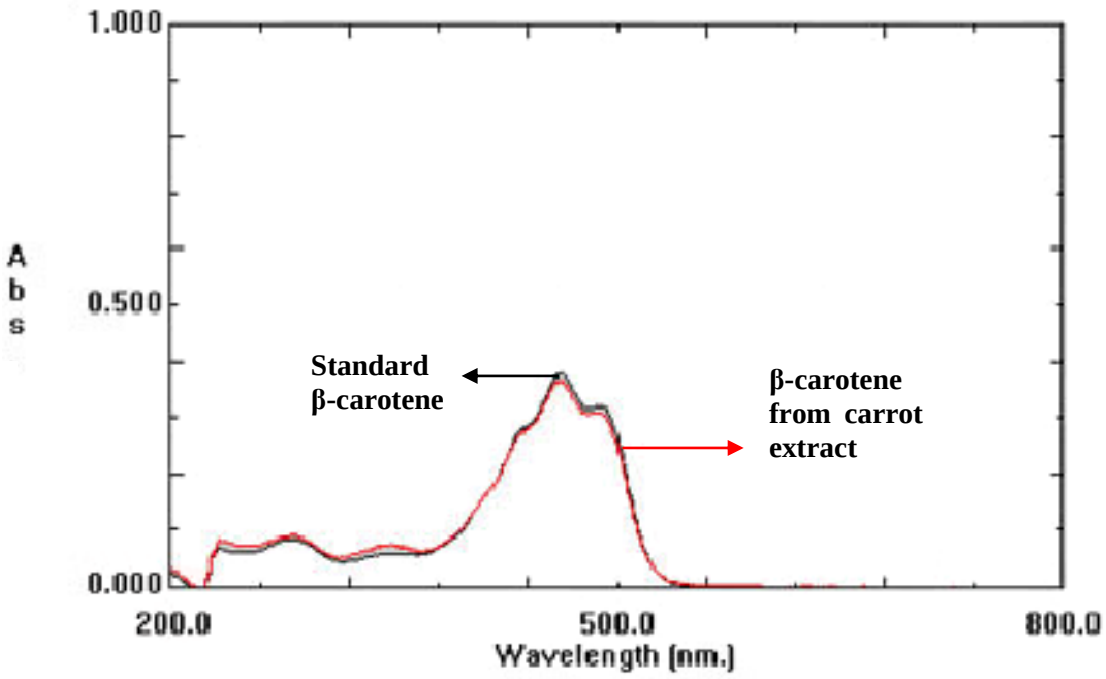


Figure 2. UV spectra of standard β-carotene and β-carotene from carrot extract.

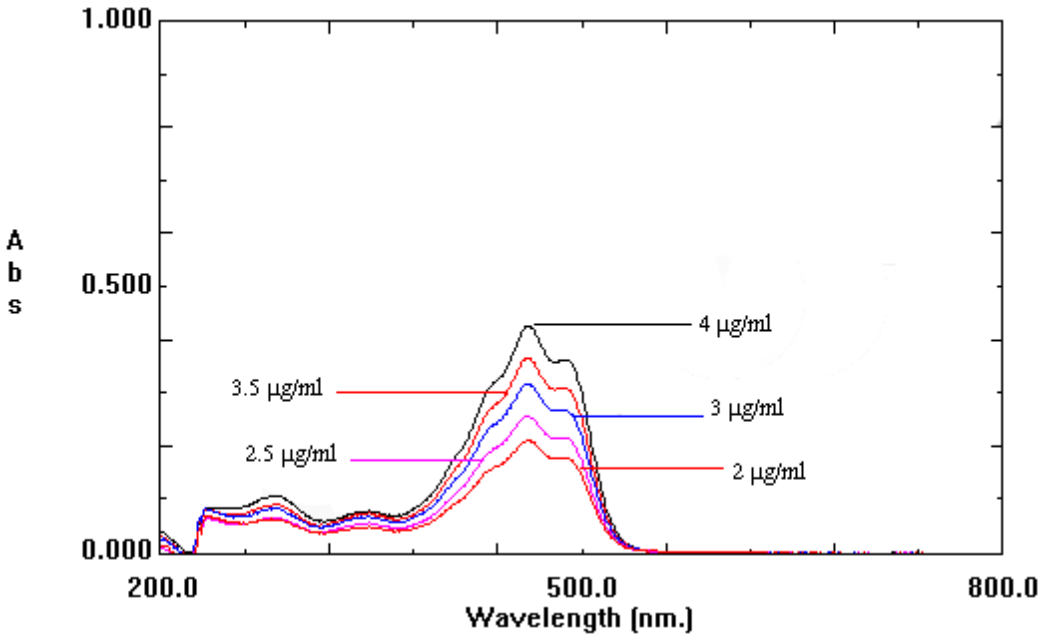
Table 2. Recovery data

Concentration (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	%R
2.0	1.92	1.85	96.3
3.0	2.92	3.01	103.1
4.0	3.83	3.88	101.3
Average	-	-	100.2
%RSD			3.43

Table 3. Application

Sample no.	β-carotene (mg/100 g carrot)	%RSD (n=3)
1	7.23	2.73
2	11.53	7.11
3	11.92	1.02
4	6.19	4.72
5	11.17	6.35
6	7.60	6.26
7	14.59	3.83

A)



B)

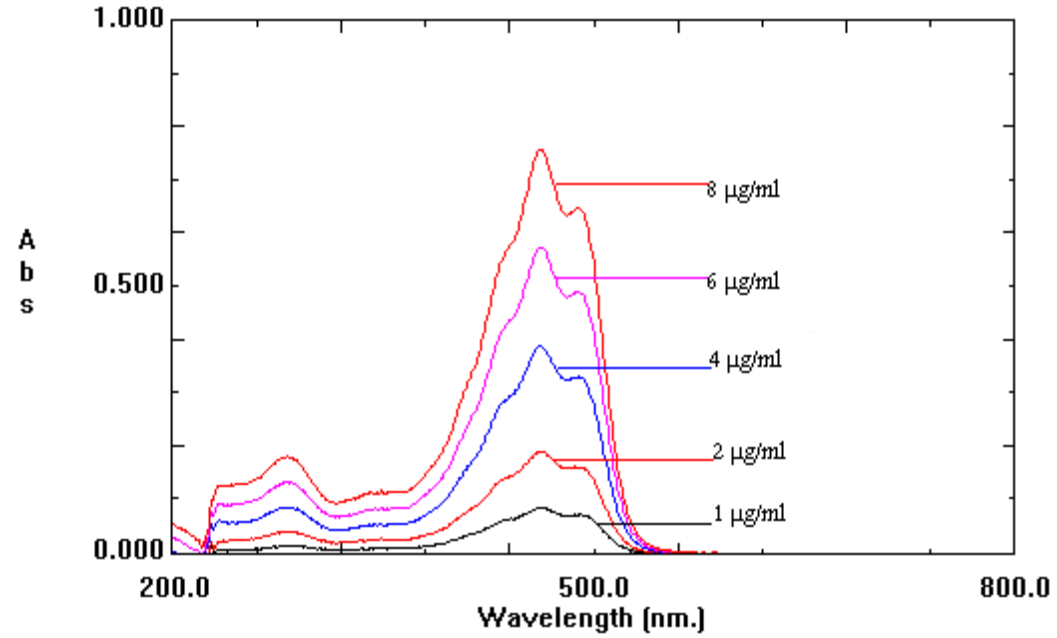
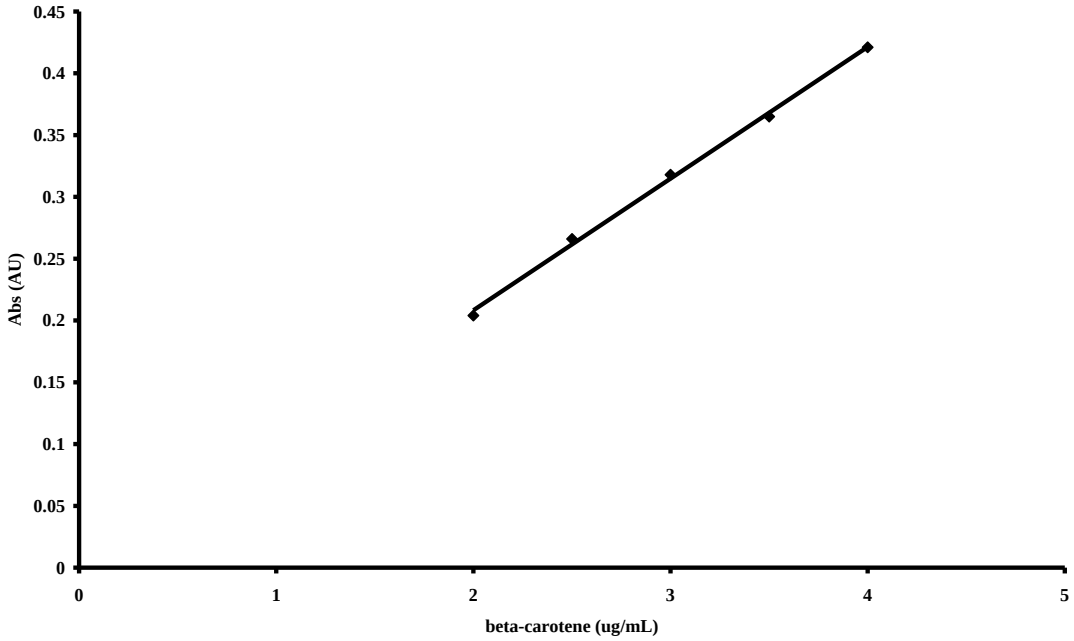


Figure 3. UV spectra of standard β-carotene at A) 2-4 and B) 1-8 µg/mL.

A)



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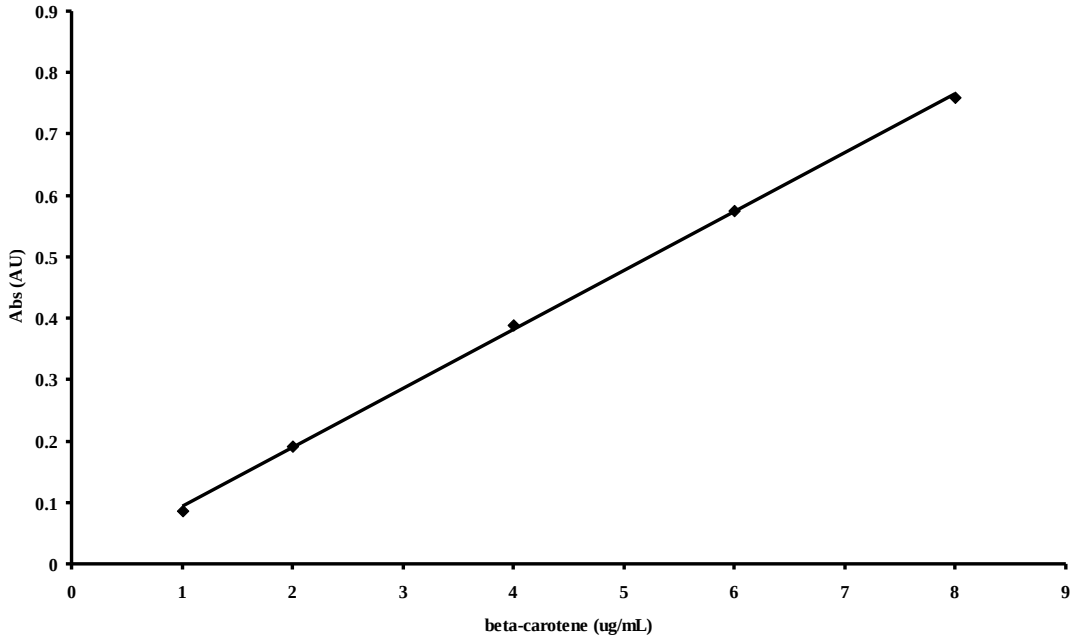

Figure 4. Calibration curves of β-carotene at A) 2-4 and B) 1-8 µg/mL.

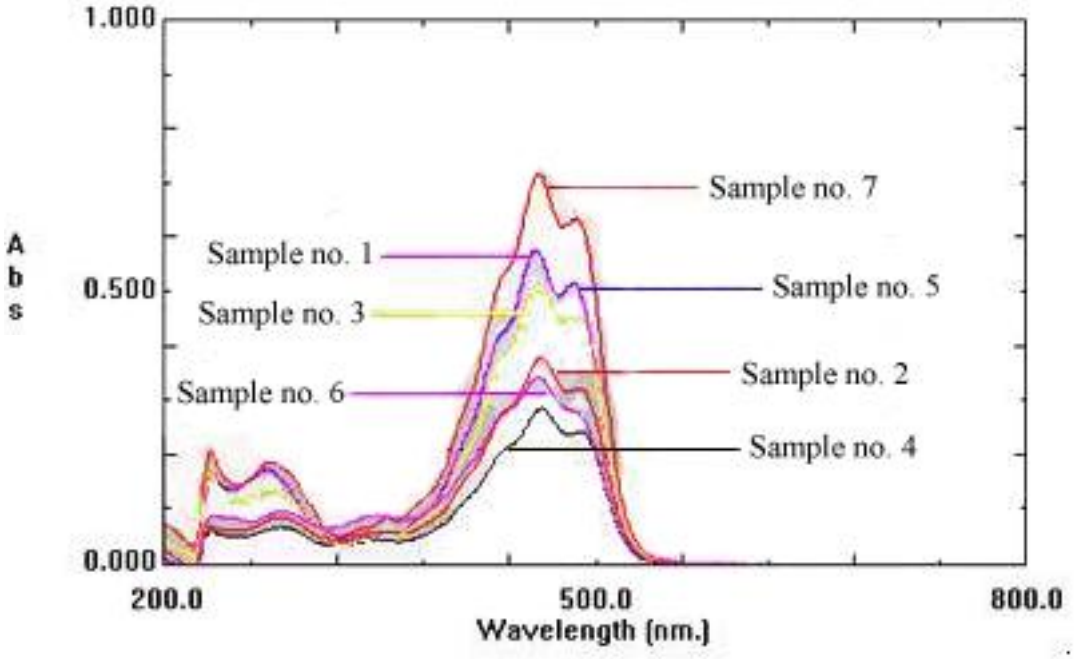

Figure 5. UV spectra of β-carotene in carrot from various sources.

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