Quantitative Determination of β-carotene Aided by Hexane / Ethanol Extraction

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Summary：Beta-carotene is an important functional ingredient in human food supply, which needed to be quantified exactly. In presented study, pure β-Carotene was used to evaluate four methods for the quantitative determination of β-carotene. The four methods included UV Spectrophotometric determination, extraction-UV Spectrophotometric determination, HPLC(High Performance Liquid Chromatography) separation and extraction-HPLC separation with C18 column. The results confirmed that all of these methods were sufficient for β-carotene quantification. The extraction with hexane/ethanol proved the recovery of HPLC separation, especially when the concentration of β-carotene was high.

Keywords: UV Spectrophotometry; HPLC; β- carotene; Quantitative Determination; extraction

Introduction

Beta-carotene is an important functional ingredient among the carotenoids family, providing vitamin A activity from vegetable sources in the human food supply [1]. It is a well-known active phytochemical with many health-promoting properties [2]. Physiologically, β-carotene had similar chemical structure with Vitamin A, could change to be vitamin A in the human body, which was considered to be the safest and most effective vitamin A precursors [3, 4]. Meanwhile, for some chronic diseases, such as atherosclerosis, cancer, cardiovascular disease, β-carotene could play a preventive role [5]. Carotene is also used as a substance to colour products such as juice, cakes, desserts, butter and margarine. However, β-carotene has poor solubility in water, sensitive to oxygen, light and temperature [2], which g greatly restricts the application of β-carotene in many fields, such as food and beverages and so on.

Quantitative determination of β-carotene accurately is particularly important in nutrition application. There were many ways to quantify β-carotene, among which, high-performance liquid chromatography and UV spectrophotometry were frequently used [1, 4]. However, for the poor solubility in water, it is difficult to measure the amount of β-carotene accurately.

If the β-carotene was diluted in appropriate solution, its concentration might be easy to quantify. Hexane/ethanol mixture was broadly used to extract β-carotene and quantitative determination [6-9]. In presented work, the hexane/ethanol extraction was combined with spectrophotometry and HPLC to improve the reliability of β-carotene quantitative determination.

Experimental

Material

Acetonitrile (HPLC grade)，Methanol (HPLC grade)，Glacial acetic acid(HPLC grade)，Tetrahydrofuran (HPLC grade), Hexane (AR), β-carotene（≥93% purity）were all purchased from Sigma (St Louis, MO, USA). Ultrapure water from water installations (M i11i-Q Lab. M illipore Co.) was used to prepare all the solutions including PBS（pH 7.0). Other regents were all of analytical grade brought from Sangon (Shanghai, China)

Instrumentation and Analytical Conditions

Chromatography was performed using a liquid HPLC system (Shimadzu, Japan) with a C_{18} silica gel chromatography column(5 μm, 4.6 mm × 250 mm) (Waters, USA) to analyze β-carotene content[10]. Two mobile phases were assayed: (A) acetonitrile/water/glacial acetic acid (90/10/2), (B) methanol/tetrahydrofuran (40/60). The mobile phase was filtered through 0.45 μm membrane filter (Millipore, USA) and then sonicated (Branson sonicator 3210, Germany) for 30 min prior to use.

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The mobile phase flow rate was 0.8 ml/min. The column temperature was 30℃ and the absorbance was read at 450 nm. For the first 5min, mobile phases contained 98% A and 2% B, then 20% A and 80% B were used in the next 5min, 100% B was used in the last 20 min during which peak ofβ-carotene appeared.

Lambda 25 UV - visible spectrophotometer（PerkinElmer, USA）was also used for β-carotene quantification with 1.0 cm quartz cell. The wavelength of 450 nm was selected for the quantitation of β-carotene against PBS or Hexane/ethanol mixture as blank.

Preparation of Standard Solutions

(a) HPLC Method. For the calibration curve, accurately weighed 25.0 mg of β-carotene was transferred to a 25mL volumetric flask and dissolved in ethanol (40℃). To give a concentration of 1 mg•mL^{-1} of the mother liquor. From this solution, other solutions with concentrations of 0.0005～0.5 mg•mL^{-1} were obtained by diluting with PBS (pH 7.0)adequate amounts in triplicate.

(b) Extraction-HPLC. For the calibration curve, accurately weighed 25.0 mg of β-carotene was transferred to a 25mL volumetric flask and dissolved in ethanol (40℃). From this solution, other solutions with concentrations of 0.005～0.3 mg•mL^{-1} were obtained by diluting with hexane amounts in triplicate.

(c) UV Method. For the calibration curve, accurately weighed 25.0 mg of β-carotene was transferred to a 25mL volumetric flask and dissolved in ethanol (40℃). From this solution, other solutions with concentrations of 0.005～0.1 mg•mL^{-1} were obtained by diluting in PBS adequate amounts in triplicate.

(d) Extraction-UV. For the calibration curve, accurately weighed 25.0 mg of β-carotene was transferred to a 25mL volumetric flask and dissolved in ethanol (40℃). From this solution, other solutions with concentrations of 0.001～0.03mg•mL^{-1} were obtained by diluting in hexane amounts in triplicate.

Preparation of Sample Solutions

For the calibration curve, accurately weighed 25.0 mg of β-carotene then transferred to a 25mL volumetric flask and dissolved in Ethanol (40℃) to give a concentration of 1mg•mL^{-1} of the mother liquor. This solution was then appropriately diluted with the PBS solvent.

The sample which detected by HPLC method and UV method diluted with the PBS solvent. However, the β-carotene detected by Extraction-HPLC method and Extraction-UV method need to extracted after diluted.

All resulting solutions were diluted with PBS solutions to obtain final concentrations within the limits of linearity for the proposed methods. Each sample was done with three parallel.

Extraction Procedure

For the assay, 1 mL samples prepared following section 2.2.2 and 2.4.4 were used. To extractβ-carotene, 1 mL hexane/ ethanol (3:1) mixture was added to the sample of same volume. After a gentle shaking, the tube was stirring (300 r/min, 5 min) to separate the organic phase from aqueous phase. The β-carotene concentration in the organic phase was quantified by HPLC and UV method at wavelength of 450 nm. And then the concentration in organic phase was transformed to concentration in aqueous phase, based on standard curve between concentration in organic phase and that in aqueous phase.

Method Validation and Comparison

The spectrophotometric and chromatographic methods were completely validated according to the procedure described in ICH guidelines Q2(R1) for validation of analytical methods. The quantitative results were statistically evaluated by Student's t-test ($p < 0.05$).

Results

Linear Range

Four methods, HPLC，extraction-HPLC, UV, extraction-UV were used to quantify β-carotene in triplicate, determining their linear ranges. For ultraviolet-visible spectrophotometry, their linearity ranges were observed to be 0.005～0.1 mg/mL and 0.001～0.03 mg/mL with correlation coefficient of 0.9997 and 0.9996, with and without extraction respectively. The calibration curves of both for HPLC method and Extraction-HPLC method, the linearity ranges were both 0.0005 ～ 0.5 mg/mL. The correlation coefficients and linearity ranges of these methods were list in Table-1.

Table-1: Correlation coefficients and linearity ranges for the quantitative determination of β-carotene.

Methods	Regression equation	R^2	Linear range(mg/mL)
HPLC	y = 1473.3x + 0.56	0.9996	0.0005～0.5
Extracted-HPLC	y = 1551.2x + 0.21	0.9999	0.0005～0.5
UV	y = 6.430x - 0.007	0.9996	0.005～0.01
Extracted-UV	y = 56.64x - 0.017	0.9997	0.001～0.03

Precision

The repeatability (intra-day and inter-day precision) of the methods was determined as shown in Table 2. Among all the methods, UV spectrophotometry had best recovery of 94-102%, its intra-day and inter-day precision (RSD) at different concentration levels were always less than 2%. The Extraction-UV method showed poorest recovery, its RSD values were less than 2% as well.

For HPLC method, excellent recoveries of β-carotene were obtained at each concentration. In terms of precision, however, the reported RSD values reflected the high precision of the proposed methods when they are applied to the assay of quantitative β-carotene. The results of Extraction-HPLC method were within 97 ± 0.5%, indicating that the proposed methods were accurate and more stable than the other three methods. But the %RSD (less variation) shows poor precision than other three methods.

Limits of Detection and Quantity

Either Extraction of not, the limit of detection and quantity would not change for both UV and HPLC. For UV method limits of detection and quantity were 1.49 ug/mL and 4.52ug/mL. For HPLC method limit of detection and quantity were 0.120ug/mL, and 0.234ug/mL, respectively. These values indicated that HPLC have better sensitivity.

Discussion

In this study，pure β-carotene was used to evaluate the methods of β-carotene quantification. Four methods, UV method，extract-UV method, HPLC and extract -HPLC were evaluated. The results showed that all four methods were linear in certain ranges. Compared to the UV method, HPLC methods showed a wide linear range in the determination of β-carotene. These results confirm that all of these methods were sufficient for β-carotene quantification [3, 11]. Combined with other measurements, such as mass spectrometric detection, the limits of detection and quantification of HPLC method would be much lower [12].

Quantification of β-carotene in this study was of good reproducibility, based on the precision. The relative standard deviation (RSD) values and the intermediate precision studies were found to be less than 5.0% for these four methods, indicating that the methods were sufficiently precise[13]. Besides, the recoveries of β-carotene were quite high at each concentration, for all proposed methods[14, 15].

It was obvious that HPLC methods had higher recovery than UV methods. But the RSD of UV method was lower than HPLC method. Considering the convenience, UV method was not a bad one. When extraction was used, the different effects were found on the two methods. Data from extraction-UV showed much lower quality than UV alone; both accuracy and RSD were worse. Situation changed on extraction-HPLC. The extraction proved the recovery, although RSD increased. When the concentration of β-carotene was high, the extraction was helpful. The main reason might be the low solubility of β-carotene in the aqueous phase[16].

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Table-2: The comparison of precision results among four methods.

Methods	Intra-day precision				Inter-day precision		
	Nominal concentration (mg/mL)	Mean concentration ±S.D.	Accuracy (% recovery)	Intraday % RSD	Mean concentration ± S.D.	Accuracy (% recovery)	Interday % RSD
UV	0.02	0.0199±0.0001	99.5	0.54	0.0189±0.0004	94.5	1.18
	0.05	0.0512±0.0002	102.4	0.61	0.0481±0.0003	96.2	1.99
Extraction-UV	0.016	0.011±0.0004	68.8	1.15	0.010±0.0005	62.5	1.53
	0.02	0.016±0.0001	80.0	1.42	0.014±0.0001	70.0	1.87
HPLC	0.05	0.0497±0.0005	99.4	0.91	0.0489±0.0006	97.8	1.19
	0.1	0.0958±0.002	95.8	1.92	0.0914±0.002	91.4	2.15
Extraction-HPLC	0.01	0.0098±0.0001	98.0	2.33	0.0097±0.0001	97.0	2.80
	0.1	0.097±0.001	97.0	2.63	0.097±0.001	97.0	3.05

References

1. J. C. Spada, L. D. F. Marczak, I. C. Tessaro and C. P. Z. Norena, Microencapsulation of Beta-Carotene Using Native Pinhao Starch, Modified Pinhao Starch and Gelatin by Freeze-Drying, *Int J Food Sci Tech*, **47,** 186 (2012).
2. R. Liang, C. F. Shoemaker, X. Q. Yang, F. Zhong and Q. R. Huang, Stability and Bioaccessibility of Beta-Carotene in Nanoemulsions Stabilized by Modified Starches, *J Agr Food Chem*, **61**, 1249 (2013).
3. L. Yonekura and A. Nagao, Intestinal Absorption of Dietary Carotenoids, *Mol Nutr Food Res*, **51** 107 (2007).
4. J. E. M. Ferreira and D. B. Rodriguez-Amaya, Degradation of Lycopene and Beta-Carotene in Model Systems and in Lyophilized Guava during Ambient Storage: Kinetics, Structure, and Matrix Effects, *J Food Sci*, **73,** C589 (2008).
5. A. V. Rao and H. L. Shen, Effect of Low Dose Lycopene Intake on Lycopene Bioavailability and Oxidative Stress, *Nutr Res*, **22** 1125 (2002).
6. Z. De-xi, Y. Biao and L. He, Determination and Analysis of β-Carotene in Berries of Different Sea Buckthorn Cultivars by Spectrophotometry, *Liaoning Agricultural Sciences*, 22 (2013).
7. S. S. Chen and M. Spiro, Kinetics of Microwave Extraction of Rosemary Leaves in Hexane, Ethanol and a Hexane plus Ethanol Mixture, *Flavour Frag J*, **10,** 101 (1995).
8. A. I. O. Barba, M. C. Hurtado, M. C. S. Mata, V. F. Ruiz and M. L. S. de Tejada, Application of a UV-Vis Detection-HPLC Method for a Rapid Determination of Lycopene and Beta-Carotene in Vegetables, *Food Chemistry*, **95** 328 (2006).
9. N. Nareswari, T. Estiasih and E. S. Murtini, Antioxidant Activity of Carotene-rich Sweet Potato Extracted with an Hexane-Ethanol Solvent Mixture, *Jurnal Teknologi Pertanian*, **7** 150 (2006).
10. S. Jian, P. Hong Xiang, D. Xin-hong, Z. En-Jun and R. Jiao-Yan, HPLC Analysis Method on β-Carotene in Sweet Potato, *Food Science and Technology*, **34** 236 (2009).
11. T. Huo, M. G. Ferruzzi, S. J. Schwartz and M. L. Failla, Impact of Fatty Acyl Composition and Quantity of Triglycerides on Bioaccessibility of Dietary Carotenoids, *J Agric Food Chem*, **55** 8950 (2007).
12. H. G. Daood, G. Bencze, G. Palotas, Z. Pek, A. Sidikov and L. Helyes, HPLC Analysis of Carotenoids from Tomatoes Using Cross-Linked C18 Column and MS Detection, *J Chromatogr Sci*, **52** 985 (2014).
13. M. Sarkar, S. Khandavilli and R. Panchagnula, Development and Validation of RP-HPLC and Ultraviolet Spectrophotometric Methods of Analysis for the Quantitative Estimation of Antiretroviral Drug, *Journal of Chromatography* B, **830,** 349 (2006).
14. A. K. Biswas, J. Sahoo and M. K. Chatli, A Simple UV-Vis Spectrophotometric Method for Determination of Beta-Carotene Content in Raw Carrot, Sweet Potato and Supplemented Chicken Meat Nuggets, *Lwt-Food Sci Technol*, **44** 1809 (2011).
15. S. M. Dhole, P. B. Khedekar and N. D. Amnerkar, Comparison of UV Spectrophotometry and High Performance Liquid Chromatography Methods for the Determination of Repaglinide in Tablets, *Pharm Methods*, **3**, 68 (2012).
16. E. J. Rousseau, A. J. Davison and B. Dunn, Protection by Beta-Carotene and Related Compounds Against Oxygen-Mediated Cytotoxicity and Genotoxicity: Implications for Carcinogenesis and Anticarcinogenesis, *Free radical biology and medicine*, **13** 407 (1992).