Validated spectrophotometric method for the analysis of total carotenoids in capsicum oil

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Abstract

Capsicum oil contained capsaicinoids and carotenoids as natural active compounds. Carotenoids are isoprenoid metabolites that are essential for life. Carotenoids are being used in pharmaceutical, nutraceutical and cosmeceutical products. The study aimed to determine total carotenoid content using spectrophotometric method. Quantitative analysis using spectrophotometric methods was selected due to rapid and reliable method. The analytical method required validation procedure for the purpose of quality control and regulatory compliance. Method validation was evaluated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), specificity and robustness of the stock standard solution. The validated method showed good linearity and range, precision, accuracy and sensitive for determination total carotenoids in capsicum oil. The spectrophotometric method also proved that it was convenient and rapid to determine total carotenoids in capsicum oil. The validated method can be applied to the analysis of carotenoids in other samples.

***Keywords*:** carotenoids, capsicum oil, spectrophotometric method, method validation

1. Introduction

Capsicum oil is extracted from dried capsicum fruit using a screw press machine, and it contains significant amounts of capsaicinoids such as capsicum oleoresin. Capsaicinoids are responsible for pungent characteristics of chili. On the other hand, carotenoids are a class of natural pigments synthesized by plants, algae, and photosynthetic bacteria. Carotenoids are essential in biological functions and have a vital role in maintaining good health. They produce antioxidant, anti-inflammatory, and antimicrobial activities (Sun, Rao, Zhou, & Li, 2022). While dietary carotenoids are good sources of provitamin A, accumulated carotenoid in the skin exhibited photoprotective properties (Zerres and Stahl, 2020). Carotenoids are sources of yellow, orange, and red color in many fruits and vegetables. Beta carotene is one of the most common carotenoids found in many fruits and vegetables. Beta carotene, beta cryptoxanthin, zeaxanthin, capsanthin and capsorubin were previously reported to be characteristics of the Capsicum species (Giuffrida et al., 2013). Figure 1 shows chemical structures of carotenoids found in Capsicum species. Capsicum oil which was obtained from cold pressing technique preserved flavor and bioactive compounds. Therefore, it gained attention to be used as ingredients and additives in food, cosmetics, and pharmaceutical industries.

There were several methods to determine carotenoids in plants, food, and dietary supplements. Although high performance liquid chromatography equipped with diode array detector is common for the analysis (Topuz & Ozdemir, 2007; Rodríguez-Rodríguez, Sánchez-Prieto, & Olmedilla-Alonso, 2020), it requires time and experience for the analysis. In addition, the recommended stationary phase in liquid chromatography for the analysis of carotenoids is carbon 30 which is not commonly used. Recent developed methods required sample extraction procedure and reversed phased carbon 18 was used. However, carotenoid quantification depended on the intended purpose and not need to characterize individual component. Spectrophotometric method is another choice that is rapid and reliable method (Luterotti, Bicanic, & Požgaj, 2006; Karnjanawipakul, Nittayanuntawech, Rojsanga, & Suntornsuk, 2010; Popescu et al., 2022). Although spectrophotometric method cannot separate *cis*- and *trans*- isomers of beta carotene, it was still a useful technique for the analysis of carotenoids in plants and dietary products. Spectrophotometric methods combined with advanced techniques, i.e., chemometrics can be applied for classification and authentication

of samples. In this study spectrophotometric method was selected to determine carotenoid content in capsicum oil. As a result, the method was validated for linearity, accuracy, precision, and specificity.

Carotenoids are soluble in variety of organic solvents; for example, beta carotene is soluble in alcohol, acetone, hexane; zeaxanthin is soluble in chloroform, and dichloromethane (Popova, 2017). In this study, isopropyl alcohol was selected as the solvent due to its less toxicity. The maximum absorption wavelength of isopropyl alcohol is 205 nm which is the lowest one compared with those of acetone, cyclohexane, dichloromethane, and hexane. Therefore, it results in less solvent effect. Capsicum oil is freely soluble in isopropyl alcohol too. Capsicum oil from Chinda Chili (*Capsicum annuum* L.) was a sample in this study.

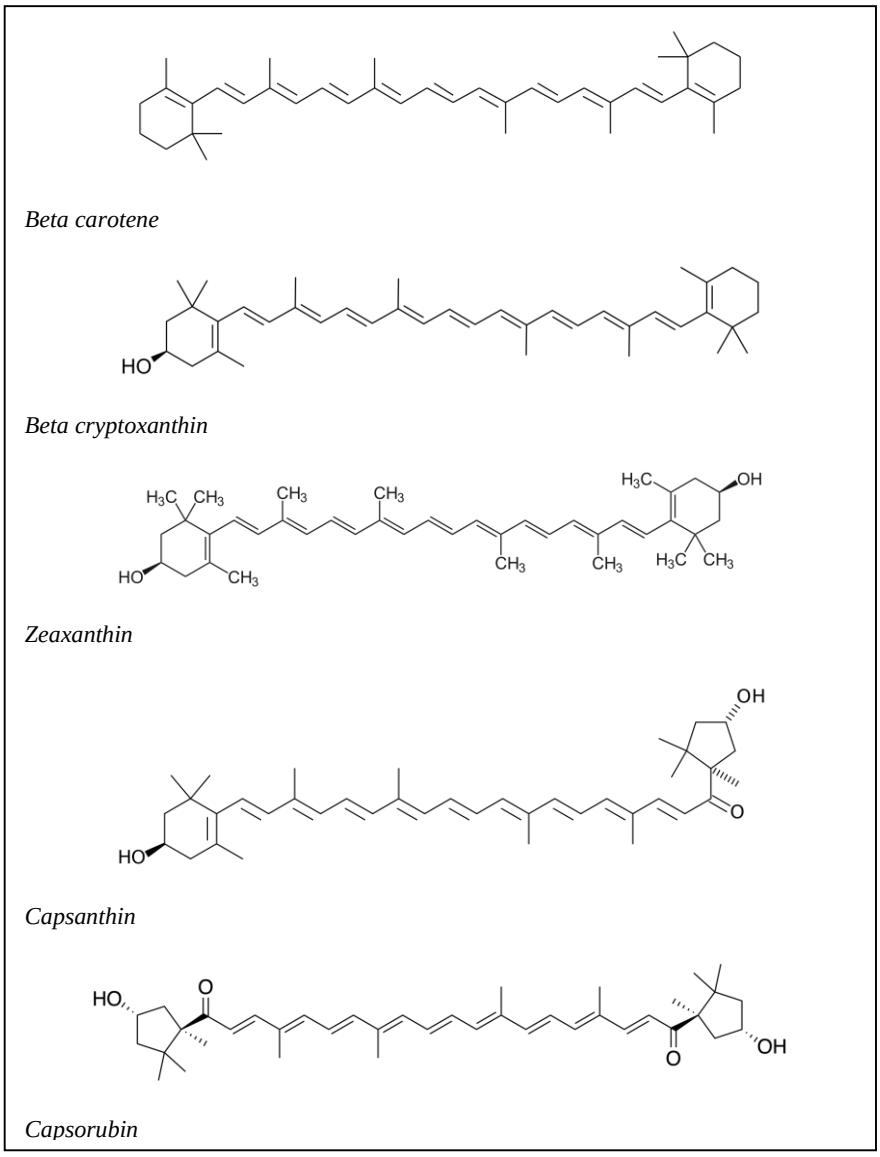

Figure 1 Chemical structures of carotenoids found in Capsicum species.

2. Objectives

The objective of the study was to validate spectrophotometric method for the analysis of total carotenoid contents in capsicum oil.

3. Materials and Methods

Chemicals and instruments

Standard beta carotene was purchased from Sigma Aldrich. Isopropyl alcohol analytical grade solvent was purchased from Burdick&Jackson, Korea. Capsicum oil was purchased from cold pressed oil group, Lopburi province, Thailand.

Absorbance was measured at the wavelength of 449 nm and spectral scan in a range of 300-600 nm on a Shimadzu UV-visible spectrophotometer model 1800.

Standard and sample preparation

Standard beta carotene for calibration curve was prepared as the stock solution at the concentration of 10 mg/mL. Then the working solutions were diluted to the concentration of 0.08 – 2.5 mg/mL in isopropyl alcohol. Capsicum oil was prepared at the concentration of 10 mg/mL and further diluted to final concentration of 0.4 mg/mL in isopropyl alcohol.

Method validation

The method was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and specificity according to ICH Q2(R1) guideline (1996). The beta carotene stock solution was kept in the refrigerator (4-8 °C) for 7 days and examined its stability and robustness of the method. The linearity of the method was carried out in a range of 0.08 – 2.5 mg/mL and range were performed at the concentration of 0.32, 0.64 and 0.96 mg/mL. Linear regression and correlation coefficient (R^2) were calculated using Microsoft Excel program. Accuracy was determined using the standard addition method of standard beta carotene at the concentration of 0.32, 0.64 and 0.96 mg/mL (n=3) and capsicum oil solution 0.4 mg/mL. Percent recovery was calculated from spiked and unspiked samples (amount found/amount added) x100). Intraday precision was determined from absorbance measurement of standard beta carotene solution at the concentration of 0.32, 0.64 and 0.96 mg/mL in triplicate on the same day while inter-day precision was measured these solutions in three different days. Repeatability was reported as percent relative standard deviation (%RSD). LOD and LOQ were calculated from (3.3 x σ)/S and (10 x σ)/S, where σ and S were standard deviation and slope of the calibration curve, respectively. Specificity was determined from spectral scan (300-600 nm) of capsicum oil solution compared with standard beta carotene solution.

4. Results and Discussion

Various solvents were examined, including acetone, cyclohexane, hexane, and isopropyl alcohol. Beta carotene and capsicum oil were completely soluble in isopropyl alcohol. Their UV spectra showed the characteristics of carotenoids without solvent interference. The UV spectrum of beta carotene was scanned from 300-600 nm, and the maximum absorption wavelength was at 449 nm. The UV spectrum of capsicum oil solution in isopropyl alcohol was overlaid, resulting in the maximum absorption at the same wavelength (Figure 2). Therefore, this wavelength was measured for analysis.

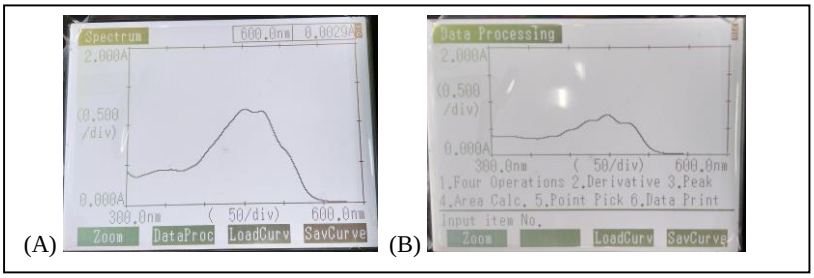

Figure 2 Spectroscopic spectrum 300-600 nm of (A) standard beta carotene and (B) capsicum oil

Quantitative analysis of total carotenoids of capsicum oil was 161.36±0.83 %w/w. Linearity was performed in a range of 0.08-2.5 mg/mL and the method showed good linearity with a regression of y=1.0211x+0.0122 (R^2=0.9999). Range of the calibration curve was obtained at 0.32, 0.64, 0.96 mg/L with acceptable linearity (y=1.0895x+0.0038, R^2=1) also. Method accuracy was obtained from the standard addition method and showed accuracy (%Recovery = 97.66-100.96) in the range of tested concentration (50% to 150%) with %RSD of 1.84. Method repeatability showed precise with %RSD of 0.000-0.014 and 2.34-3.34 for intraday and inter-day precision, respectively. LOD and LOQ were 0.1 and 0.22 mg/mL, respectively. LOQ also was agreed with Beer-Lambert law with the absorbance of 0.215±0.000. The spectrophotometric method resulted in linear, accurate, and precise for the estimation of total carotenoids in capsicum oil. Spectrophotometric method validation was summarized and shown in Table 1.

Table 1 Analytical method validation

Parameters	**Results**				**Criteria**
Linearity	y = 1.0211x + 0.0122				R^2 > 0.995
(0.08 – 2.5 mg/mL)	R^2 = 0.9999				
Range	y = 1.0895x + 0.0038				R^2 > 0.995
(0.32 – 0.96 mg/mL)	R^2 = 1				
Accuracy					95 – 105%
Conc. (mg/mL)					
0.32	97.66 ± 4.38				
0.64	100.96 ± 1.29				
0.96	100.71 ± 1.60				
Repeatability	Intraday precision (%RSD)			Inter-day precision	%RSD
Conc. (mg/mL)	Day 1	2	3		< 3.7%
0.32	0.014	0.001	0.002	3.34	intraday
0.64	0.000	0.001	0.000	2.34	< 6%
0.96	0.000	0.000	0.000	2.55	Inter-day
LOD (mg/mL)	0.10				
LOQ (mg/mL)	0.22				

Table 2 shows that the standard beta carotene stock solution stored in the refrigerator at 4-8 °C for 7 days can be used for preparation of the working solutions. The absorbance of the working solutions (0.32, 0.64, 0.96 mg/mL) slightly decreased, and they were not significantly different compared with those absorbances of the solution prepared on the first day.

Table 2 Robustness of the method

Conc. (mg/mL)	**Day 1**		**Day 7**	
	Absorbance (449 nm)	**%RSD**	**Absorbance (449 nm)**	**%RSD**
0.32	0.354±0.005	1.38	0.309±0.003	0.94
0.64	0.699±0.000	0.04	0.619±0.001	0.09
0.96	1.051±0.000	0.02	0.933±0.001	0.08

In the preliminary process some organic solvents were tested, and isopropyl alcohol was selected. The validated method did not require the extraction step for sample preparation. Previous study reported carotenoid content in red chili powder was 602.06 µg/g (0.06%w/w) (Giuffrida et al., 2014). The content was slightly lower than carotenoid content of the ethanolic extract of Chinda chili powder (0.82 ±0.06%w/w). In addition, the carotenoid content was much lower than those of capsicum oil (161.36±0.83 %w/w). Carotenoids are lipophilic property; therefore, they are much more soluble in lipid phase which is the main part in squeezed capsicum oil. Another study reported the carotenoid content in fresh red, orange, and yellow pepper in the range of 146.5-3343.4 µg/100 g fresh pepper (Pugliese et al., 2013). Additionally, capsanthin

was one of the prominent carotenoids found in these peppers. Carotenoid contents were varied dependent on chili or pepper species.

5. Conclusion

The spectrophotometric method was validated in terms of linearity, accuracy, and precision. Carotenoid content in capsicum oil was 161.36±0.83 %w/w. Beta carotene stock solution should be kept in dark container in the refrigerator and stable for one week. The analytical method can be applied to analyze carotenoids in other capsicum samples i.e., capsicum oleoresin. The validated method can be used as a routine method for the analysis of total carotenoids in capsicum oil.

6. Acknowledgements

The authors sincerely acknowledge College of Pharmacy, Rangsit university for the laboratory instruments. The authors greatly appreciate professional pharmacy students for their help in these experiments (Training rotation academic year 2022).

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