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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-8050

Simultaneous Analysis of Fat-Soluble Vitamins in Beverages Using Triple Quadrupole LC-MS/MS

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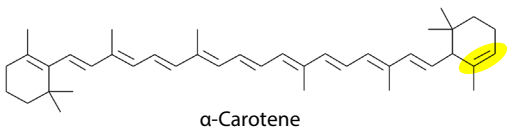
User Benefits

- Rapid and simultaneous determination of 13 fat-soluble vitamins A, D, E, and K.
- High-sensitivity analysis using a triple quadrupole mass spectrometer.
- Individual quantification of α-carotene and β-carotene were achieved.

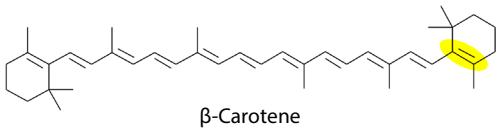
■ Introduction

Vitamins play an important role for maintaining human body functions in good condition and need to be obtained from food because they cannot be synthesized in the body. Vitamins A, D, E, and K, which are not soluble in water, are called fat-soluble vitamins. Vitamin A mainly refers to retinol, which has the function of keeping eyes and skin healthy and enhancing immunity. On the other hand, α-carotene and β-carotene are called provitamin A because they are converted to retinol in the body. The conversion rates of those two compounds are different, and the bioavailabilities of α- and β-carotene are estimated to be 1/24 and 1/12 compared to that of retinol, respectively[1]. To evaluate the activity of vitamin A, individual quantification of α- and β-carotene are necessary. It is difficult to separate these compounds with LC-MS/MS in the past since these are isomers and therefore have very similar structures (Fig. 1).

In this paper, we introduce simultaneous quantification of fat-soluble vitamins including α- and β-carotene by optimizing LC conditions. We performed individual quantification of α- and β-carotene and recovery tests on vegetable juice using this method. As α- and β-carotene do not have ester compounds, alkaline hydrolysis treatment, which is usually needed in sample preparation, can be omitted. Good recovery rates were obtained with a simple extraction operation.



α-Carotene



β-Carotene

Fig. 1 Structure of α- and β-carotene

■ Experimental

5 g of anhydrous sodium sulfate was added to 1 g of commercially available vegetable juice, then ethanol was added and the mixture was shaken for 5 minutes to be subjected to centrifugation at 4,000×*g* for 5 minutes, then obtained supernatant was collected. The above extraction process was repeated twice with the remaining sediment. The collected supernatants were combined and made up to 40 mL with ethanol. After diluting the extraction solution 100 times with ethanol, the supernatant was collected as the extraction sample after centrifuging at 15,000 rpm for 10 minutes.

The analysis was carried out using a system that combined a triple quadrupole mass spectrometer LCMS-8050 and an ultra-high-speed liquid chromatograph Nexera™ X3. The LC-MS/MS analytical conditions and MRM conditions are shown in Tables 1 and 2, respectively.

Table 1 LC-MS/MS conditions

[HPLC conditions] (Nexera X3)	
Column	: Kinetex XB-C18 (100 mm x 3.0 mm I.D., 1.7 µm)
Mobile phase A	: Methanol
Mobile phase B	: Ethanol
Flow rate	: 0.5 mL/min
Gradient program	: B conc. 15% (0-7 min) - 100% (7.01-12 min) - 15% (12.01-14 min)
Column temp.	: 40°C
Injection volume	: 5 µL
[MS conditions] (LCMS-8050)	
Ionization	: APCI
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
DL temp.	: 200°C
Interface temp.	: 300°C
Heat block temp.	: 200°C

Table 2 MRM conditions

Compound	Ret. Time (min)	Polarity	Transition	CE (V)
Retinol	1.16	+	269.20> 77.10	-53
α-Carotene	6.35	+	537.45>123.15	-21
β-Carotene	6.71	+	537.45>119.10	-39
β-Cryptoxanthin	2.57	+	553.45>119.00	-33
Ergocalciferol	1.75	+	397.35> 69.00	-24
Cholecalciferol	1.80	+	385.35>259.20	-14
α-Tocopherol	1.98	-	429.30>163.10	30
β,γ-Tocopherol	1.81	-	415.35>149.10	27
δ-Tocopherol	1.66	-	401.25>135.00	30
Phylloquinone	2.96	+	451.45>187.10	-27
Menaquinone-4	2.03	+	445.40>187.10	-22
Menaquinone-7	5.94	+	649.60>187.10	-30

Nexera™ X3 and LCMS-8050

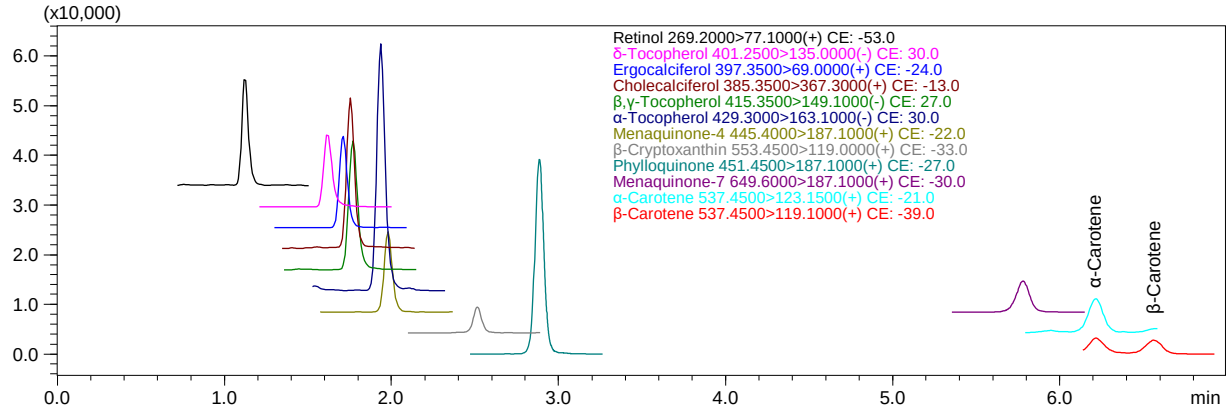


Fig. 2 Simultaneous analysis of 13 fat-soluble vitamins

■ Simultaneous analysis of 13 fat-soluble vitamins

The quantification range and repeatabilities of the standard solution of the 13 fat-soluble vitamins are shown in Table 3, and typical MS chromatograms are shown in Fig. 2. The calibration curves for α- and β-carotene are presented in Fig. 3. Accurate and wide-range quantification was achieved.

Table 3 Quantification range and repeatability

Compound	Quantification range (ng/mL)	Repeatability (%, N=5)	
		1 ppb	5 ppb
Retinol	0.1 - 1000	9.8	3.1
α-Carotene	0.5 - 1000	6.9	2.7
β-Carotene	0.5 - 0500	8.5	3.7
β-Cryptoxanthin	0.5 - 1000	3.1	1.6
Ergocalciferol	0.1 - 1000	4.2	1.7
Cholecalciferol	0.5 - 1000	9.6	4.0
α-Tocopherol	0.5 - 1000	6.9	1.5
β,γ-Tocopherol	0.1 - 1000	2.0	3.8
δ-Tocopherol	0.1 - 1000	7.5	5.5
Phylloquinone	0.5 - 1000	3.3	1.7
Menaquinone-4	0.5 - 1000	9.5	2.9
Menaquinone-7	0.1 - 1000	3.2	2.0

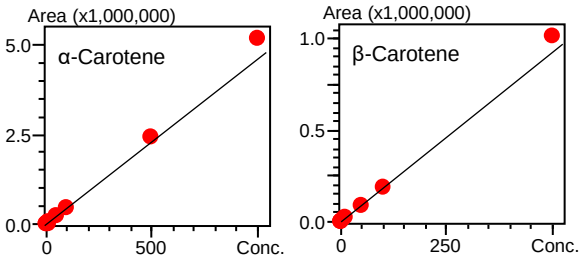


Fig. 3 Calibration curves of α-and β-carotene

■ Quantification of α- and β-carotene

Quantification of α- and β-carotene in two types of commercial vegetable juice was performed. The results are shown in Table 4. The obtained concentrations were within the range of the nutritional information declared by the manufacturer.

Table 4 Determination of α- and β-carotene in vegetable juice

Compound	Vegetable juice a (μg/mL beverage)	Vegetable juice b (μg/mL beverage)
α-Carotene	20.3	15.7
β-Carotene	40.5	56.3

■ Ion suppression effect of α- and β-carotene

Quantification of α- and β-carotene was performed by adding them into the extracted solution of two types of commercial vegetable juice, and ion suppression effect was investigated. The results are shown in Tables 5 and 6. Good recovery rates were obtained for α- and β-carotene in both types of vegetable juice.

Table 5 Recovery rate (%) of α- and β-carotene in vegetable juice a

Compound	Spiked conc. (μg/mL in vial)		
	2	5	10
α-Carotene	96.8	98.7	103.1
β-Carotene	112.9	97.4	99.9

Table 6 Recovery rate (%) of α- and β-carotene in vegetable juice b

Compound	Spiked conc. (μg/mL in vial)		
	2	5	10
α-Carotene	103.0	109.6	108.3
β-Carotene	68.5	101.1	102.9

■ Summary

Simultaneous quantification of 13 fat-soluble vitamins was performed using LC-MS/MS. This method enables individual quantification of α- and β-carotene. Quantification of α- and β-carotene in vegetable juice was performed using a simple and convenient sample preparation method without saponification. Ion suppression effect was examined, and good recovery rates were obtained.

<Reference>

1) Ministry of Health, Labour and Welfare "Dietary Reference Intakes for Japanese (2020)"

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