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# Application News

## No.L491

High Performance Liquid Chromatography

# Comprehensive 2D Separation of Carotenoids in Red Chili Pepper by the Nexera-e System

Carotenoids are naturally occurring organic pigments that are divided into two classes, carotenes, consisting only of carbon and hydrogen, and xanthophylls, which contain oxygen. Carotenoids are rich in double bonds, and therefore have received much attention in recent years as antioxidants, which are known for their disease preventive properties, including lifestyle-related diseases.
The extensive range of carotenoids found in foods makes it difficult to conduct simultaneous separation and quantitation by conventional HPLC. However, the Nexera-e comprehensive two-dimensional LC is particularly suited for such analyses. Here, carotenoids extracted from red chili pepper were subjected to two-dimensional analysis, in which micro-scale separation was conducted in the first stage using normal phase conditions, and separation using reversed phase conditions was tried in the second dimension. For detection, a combination of a photodiode array (PDA) connected to the LCMS-8030 triple quadrupole mass spectrometer was used. Because the separation modes, normal and reversed phases, differ in the first and second dimensions, this might be considered a two-dimensional LC method by which the greatest orthogonality possible is obtained.

## Comprehensive Separation of Carotenoids Detected by the Photodiode Array Detector

Use of the Nexera-e with a photodiode array detector (PDA) permits the separation of complex coexisting substances and detection at the optimal wavelength in a single analysis. Fig. 1 shows a comprehensive two-dimensional representation of the separation pattern (absorption wavelength = 450 nm) generated using the specialized two-dimensional analysis software, ChromSquare.
By combining the first-dimension cyano column and the second-dimension ODS column, 10 groups of substances, including hydrocarbons, monool esters, diol diesters, diol monoketo diesters, diol diketo diesters, diol mono epoxide monoesters, free monools, diol monoketo monoesters, diol diketo mono esters, and polyoxygenated free xanthophylls were separated according to class based on molecular polarity, and the component separation was verified based on the hydrophobicity of their respective fatty acid residues.

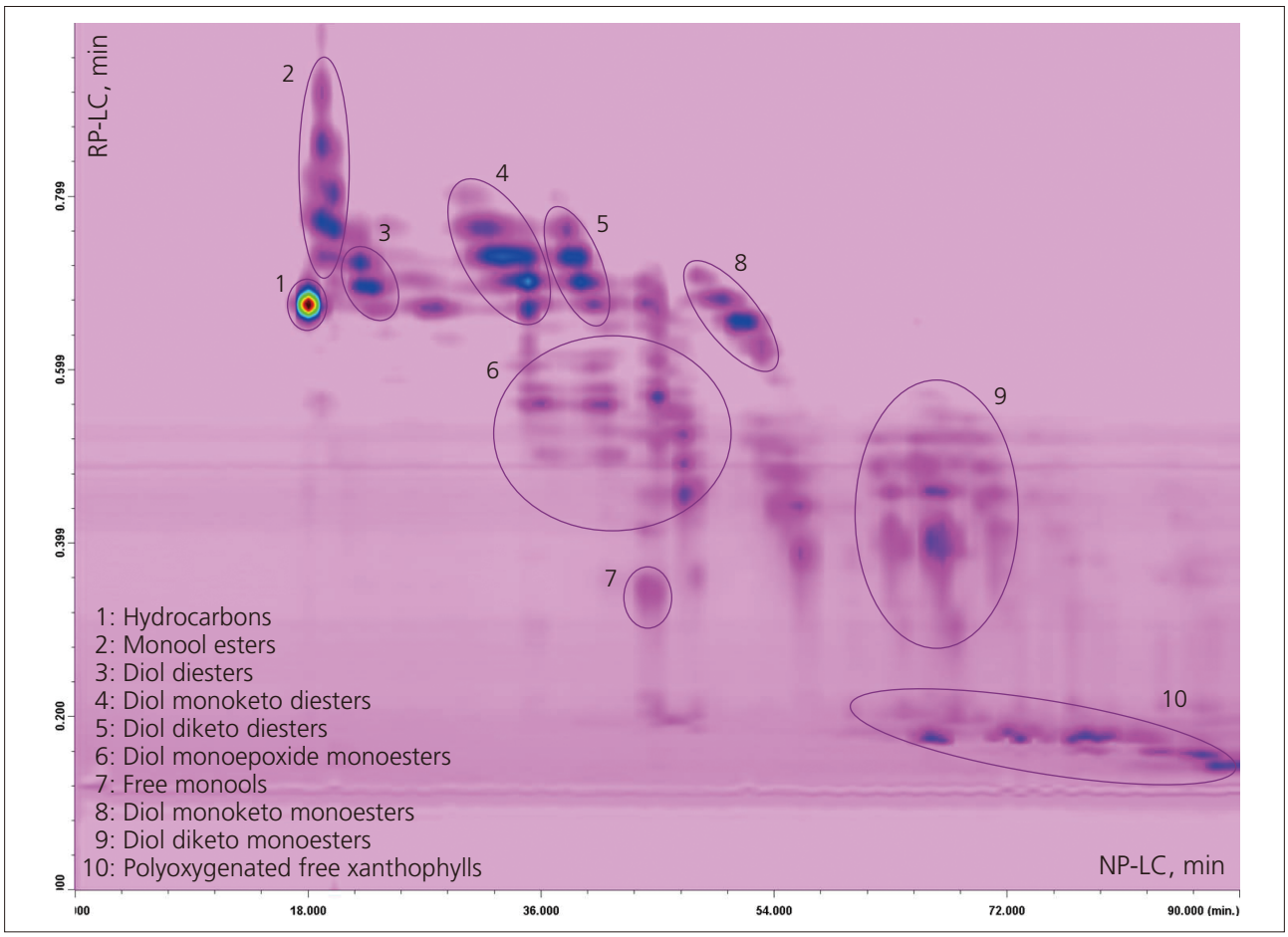


Fig. 1 2D Plot of Carotenoids Using ChromSquare Software

## Quantitation of β-Carotene in Red Chili Pepper by LC/MS/MS

The analytical conditions are shown in Table 1, and the sample pretreatment conditions are shown in Fig. 2. β-carotene, which is a precursor of vitamin A, was detected in the two-dimensional separation of the carotenoids. Quantitation was then conducted using the LCMS-8030 triple quadrupole mass spectrometer. Both high sensitivity and high selectivity can be obtained using MRM analysis, and further, reduced ion suppression can be expected with the two-dimensional separation obtained with the Nexera-e.

Fig. 3 shows the two-dimensional separation data of β-carotene obtained from DUIS-positive mode MRM analysis of the calibration curve, and Fig. 4 shows the linearity of the three values (blobs) in the range of 0.01 to 10 mg/L, which correspond to the peak volumes used for quantitation. The correlation coefficient (r) = 0.998976 indicates results with good linearity. The quantitative result for β-carotene present in red chili pepper was calculated as 1.22 mg/L based on the concentration in the final sample following extraction.

**Table 1 Analytical Conditions**

| | |
|---|---|
| 1D Colum | : Ascentis Cyano (250 mm L. × 1.0 mm I.D., 5 µm) |
| Mobile Phase | : A; Hexane<br>B; Hexane/Butylacetate/Acetone = 80/15/5 (v/v/v) |
| Flowrate | : 0.02 mL/min |
| Time Program | : B Conc. 0 % (0.01 min) → 0 % (5 min) → 100 % (65 min) → 100 % (75 min) → 0 % (76 min) |
| Column Temp. | : 30 °C |
| Injection vol. | : 2 µL |
| Loop vol. | : 20 µL |
| 2D Column | : Ascentis Express C18 (30 mm L. × 4.6 mm I.D., 2.7 µm) |
| Mobile Phase | : A; acetnitrile<br>B; 2-propanol |
| Flowrate | : 4 mL/min (0.8 mL/min split for MS) |
| Time Program | : B Conc. 0 % (0.01 min) → 50 % (0.17 - 0.54 min) → 80 % (0.54 - 0.93 min) → 30 % (0.94 min) → STOP (1 min) |
| Detector | : SPD-M30A Photo diode array detector (standard cell, wave length = 450 nm)<br>Shimadzu LCMS-8030 (DUIS positive mode, targeted β-carotene MRM transition: *m/z* 536.40 > 444.30) |

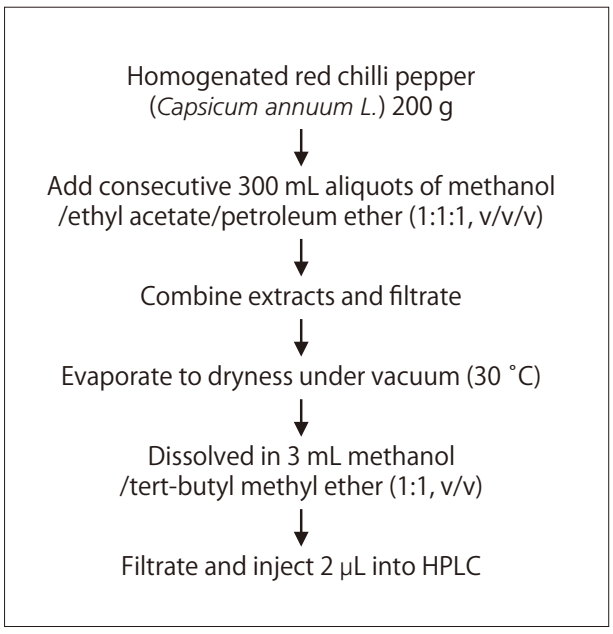


**Fig. 2 Sample Preparation**

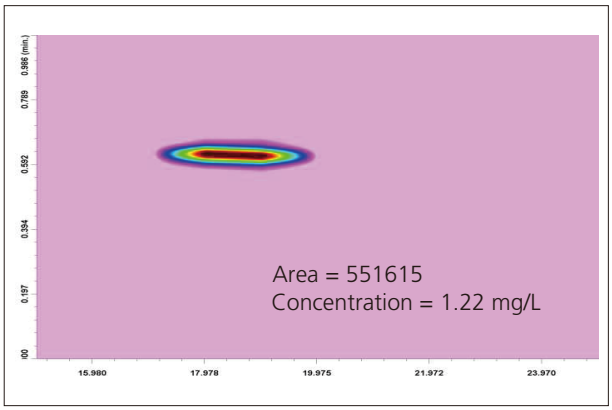


**Fig. 3 2D Plot of β-Carotene**

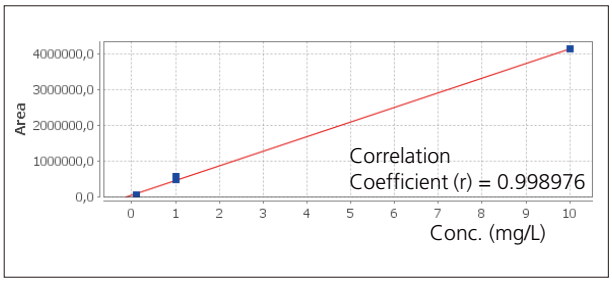


**Fig. 4 Linearity of Calibration Curve for β-Carotene**

Data provided by University of Messina Prof. Luigi Mondello and Chromaleont S.r.l.

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