Distinguishing between isomeric neoxanthin and violaxanthin esters in yellow flower petals using liquid chromatography/photodiode array atmospheric pressure chemical ionization mass spectrometry and tandem mass spectrometry

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Rationale: Liquid chromatography/photodiode array atmospheric pressure chemical ionization mass spectrometry (LC/PDA-APCI-MS) is used for the analysis of various carotenoid pigments in plants. Among them, it is difficult to distinguish between the isomeric violaxanthin/neoxanthin esters.

Methods: The yellow pigments of tomato petals were extracted with acetone, and the extracts were kept at −30°C to allow the contaminating triacylglycerols to settle out physically. The supernatants were analyzed using LC/PDA-APCI-MS with a high-resolution orbitrap mass spectrometer for their exact masses. The expected carotenoid esters were calculated with the combination of carotenoids and fatty acids, and they were matched with the experimental exact masses. The fatty acid structures in the carotenoid esters were also identified using collision-induced dissociation (CID) tandem mass spectrometry (MS/MS). The isomeric violaxanthin/neoxanthin esters were distinguished using CID MS/MS from their in-source dehydrated product ions as pseudoprecursor ions.

Results: The in-source dehydrated ions \([M - H_2O + H]^+\) of neoxanthin diesters predominated over their protonated molecules \([M + H]^+\) in LC/MS. By contrast, the protonated molecules of violaxanthin diesters predominated. The 92 u loss product ions \([M - H_2O - C_7H_8 + H]^+\) were observed from the dehydrated violaxanthin diesters, but they were not generated from the dehydrated neoxanthin diesters in the CID MS/MS of their dehydrated pseudoprecursor ion \([M - H_2O + H]^+\).

Conclusions: The allene allyl carbocation in neoxanthin diesters was generated from dehydration after preferential protonation at the hydroxy group. The epoxide group of violaxanthin diesters opens easily after protonation; however, the dehydration did not proceed at this stage. The 92 u loss of \(C_7H_8\) was explained by an intramolecular \([2 + 2]\) cycloaddition, which proceeded preferentially in dehydrated violaxanthin
diesters because the carbocations in the dehydrated species were conjugated to the polynene and those double bonds were depolarized during CID MS/MS. Therefore, the isomeric neoxanthin/violaxanthin diesters were distinguished using LC/PDA-APCI-MS and MS/MS. This method was a practical and useful method of profiling the carotenoid esters of the yellow petals.

1 | INTRODUCTION

Carotenoids are C_{40} isoprenoid pigments of yellow, orange, to red colors in fruits and flowers. The carotenoid structural varieties are generated with or without epoxide, hydroxy, and keto groups (as shown in Figure 1), which is the biosynthesis pathway of carotenoids from lycopene in which two C_{20} geranylgeranyl diphosphates (GGPP) are coupled and four C_{5} isopentenyl diphosphates are condensed to form C_{20} GGPP. The majority of carotenoids in flower petals are yellow xanthophylls, such as lutein, β-cryptoxanthin, and zeaxanthin (Figure 1). Epoxide xanthophylls such as violaxanthin, antheraxanthin, neoxanthin, and lutein-5,6-epoxide are also common. Xanthophylls impart pale yellow and deep yellow to orange colors to flowers, depending on the carotenoid content in the petals.

Carotenoids and carotenoid esters in fruits and flowers are analyzed and profiled using high-performance liquid chromatography (HPLC)/photodiode array (PDA) atmospheric pressure chemical ionization (APCI) mass spectrometry (MS). Those compounds are separated from each other using LC, and their ultraviolet–visible (UV–vis) absorption spectra with the PDA detector indicate the carotenoid structures because of their conjugated double bonds. The hyphenated MS and MS/MS measurements with LC can analyze their chemical composition formulas, the carotenoid skeletal structures, and the fatty acid structures of the carotenoids and carotenoid esters. However, there are some challenging issues of the structural elucidation of the carotenoid esters: there are fewer standards, the carotenoids have low ionization efficiency, and there are large amounts of the contaminating triacylglycerols (TAGs) that hinder the MS detection and analysis in the extracted samples from fruits and flowers.

![Biosynthesis pathway of carotenoids from lycopene in plants](image)

**Figure 1** Biosynthesis pathway of carotenoids from lycopene in plants
Saponification is routinely performed for carotenoid analysis to hydrolyze complex esters and to remove TAGs and chlorophylls.9–11 Free-form carotenoids are conveniently analyzed and identified using LC/PDA-MS and MS/MS with their authentic standards. However, there is no fatty acid information on the carotenoid fatty acid esters after saponification treatment. Lipid-removing pretreatments are useful to detect carotenoid esters, which are a physical separation of lipids in cryopreservation samples and using an open column of MgO–diatomaceous earth (two-step cleanup method).2

In this study, we used Fourier transform high-resolution orbitrap MS measurement to overcome the contamination by TAGs and to identify the correct structures of the carotenoids and their esters from their high-resolution exact mass data. Moreover, it is a challenging issue to distinguish between the isomeric neoxanthin and violaxanthin esters with LC/PDA-MS/MS. We focused our attention on distinguishing between them with the 92 u loss product ions [M – H₂O – 92u + H]⁺ as their identical product ions in the CID MS/MS spectra from the in-source dehydrated product ions as a pseudoprecursor ion.

2 MATERIALS AND METHODS

2.1 Materials

Methanol (HPLC grade), tert-butyl methyl ether (MTBE, HPLC grade), and acetone (HPLC grade) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Tomato seedlings were purchased from a local plant shop and grown up to flowering in a home garden. Yellow Mandevilla (Mandevilla sanderi) flowers (Opale “Citrine”) were obtained from SAS Lannes et fils (Malause, France).

Figure 2 The PDA chromatograms at 450 nm of the tomato flower acetone extracts (a). The mass chromatograms of neoxanthin/violaxanthin diesters correspond to C₆₈H₁₀₉O₆ at m/z 1021.80–1021.84 (b), C₇₀H₁₁₃O₆ at m/z 1049.83–1049.87 (c), and C₇₂H₁₁₇O₆ at m/z 1077.86–1077.90 (d). The peaks labeled with asterisks were not from carotenoids.
2.2 | Acetone extraction of yellow pigment component for LC/PDA-MS

Freeze-dried petals (30.0 mg) in 1.0 mL acetone were treated with ultrasonic wave irradiation for 20 min at 25°C (AS52GTU ultrasonic cleaner; AsOne Corp., Osaka, Japan) to extract yellow pigment compounds. The extracted solvent was kept at −30°C for 1 h to settle out contaminating TAGs physically. Finally, the supernatant was filtered with a 0.45 μm filter (solvent type Cosmonice Filter S 0.45 μm; Nacalai Tesque, Inc., Kyoto, Japan).

2.3 | LC/PDA analysis

LC/PDA analysis was conducted using a Nexera HPLC system composed of LC-30AD pumps and a PDA detector for 200–800 nm (Prominence SPD-M20A; Shimadzu Corp., Kyoto, Japan). A Develosil C30-UG-3 reversed-phase column (2.0 mm × 100 mm; Nomura Chemical Co., Ltd, Aichi, Japan) was used at a flow rate of 0.4 mL/min. The LC column was eluted with the gradient solutions of two mobile phases: mobile phase A was a mixture of methanol and water (80:20, v/v) and mobile phase B was a mixture of MTBE, methanol, and water (78:20:2, v/v/v), which were developed for PDA spectra to reduce the solvent absorption itself. The gradient program was as follows: 0 min, 0% B; 15 min, 100% B; 20 min, 100% B; 25 min, 0% B.

2.4 | APCI-MS and CID MS/MS analyses

APCI-MS and CID MS/MS analyses were conducted using an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA) equipped with an APCI source. The MS and MS/MS spectra were recorded for m/z 150 to 2000 in the positive-ion mode at 60,000 resolution. The peak width of CID MS/MS was m/z 3.0, and the collision energies were changed from 15 to 45 (arbitrary unit specific to the device).

The mass chromatograms were drawn with the narrow window 0.04 u from the calculated exact masses of the expected carotenoid diesters in the combination of carotenoids and fatty acids summarized in the supporting information-1 (SI-1).

![Figure 3](image-url) The LC/APCI-MS spectra of neoxanthin/violaxanthin diesters observed at 21.5 min as peak 6 (a), at 21.7 min as peak 7 (b), at 22.0 min as peak 8 (c), at 22.3 min as peak 9 (d), at 22.6 min as peak 10 (e), and at 22.8 min as peak 11 (f).
FIGURE 4  CID MS/MS spectra of neoxanthin/violaxanthin diesters observed at m/z 1021.8 at 21.5 min as peak 6 (a), at m/z 1049.8 at 21.7 min as peak 7 (b), at m/z 1077.9 at 22.0 min as peak 8 (c), at m/z 1021.8 at 22.3 as peak 9 (d), at m/z 1049.8 at 22.3 min as peak 10 (e), and at m/z 1077.9 at 22.8 min as peak 11 (f). The precursor ions were present as their protonated molecules [M + H]+. The collision energy was set at 30 (arbitrary unit specific to the device).

TABLE 1  Assignment of peaks 1–11 in the LC/PDA-MS spectra

| Peak no. | Retention time (min) | λmax (nm) | Observed m/z | Chemical composition | Error (ppm) | Compound |
|----------|----------------------|-----------|--------------|----------------------|-------------|----------|
| 1        | 18.8                 | 417, 440, 468 | 783.5927     | C52H79O5             | 0.61        | Neoxanthin monolaurate |
| 2        | 19.2                 | 417, 440, 468 | 811.6224     | C54H83O5             | 1.12        | Neoxanthin monomyristate |
| 3-1      | 19.6                 | 417, 440, 468 | 783.5911     | C52H79O5             | 1.39        | Violaxanthin monolaurate |
| 3-2      | 19.6                 | 418, 439, 468 | 839.6534     | C56H87O5             | 1.71        | Neoxanthin monopalmitate |
| 4        | 19.8                 | 417, 440, 468 | 811.6219     | C54H83O5             | 1.94        | Violaxanthin monomyristate |
| 5        | 20.2                 | 418, 439, 468 | 839.6538     | C56H87O5             | 1.17        | Violaxanthin monopalmitate |
| 6        | 21.5                 | 418, 441, 470 | 1021.8192    | C64H169O6            | 2.60        | Neoxanthin dimyristate |
| 7        | 21.7                 | 418, 440, 469 | 1049.8486    | C70H195O6            | 4.32        | Neoxanthin myristate–palmitate |
| 8        | 22.0                 | 417, 439, 468 | 1077.8790    | C72H117O6            | 5.44        | Neoxanthin dipalmitate Neoxanthin myristate–stearat e |
| 9        | 22.3                 | 419, 438, 467 | 1021.8198    | C68H109O6            | 2.06        | Violaxanthin dimyristate |
| 10       | 22.6                 | 419, 438, 467 | 1049.8499    | C70H195O6            | 3.07        | Violaxanthin myristate–palmitate |
| 11       | 22.8                 | 419, 438, 466 | 1077.8807    | C72H117O6            | 3.49        | Violaxanthin dipalmitate Violaxanthin myristate–steарат e |
3 | RESULTS AND DISCUSSION

3.1 | LC/PDA-APCI-MS

The acetone extract of tomato petals was subjected to analysis by LC/PDA-APCI-orbitrap MS. Abundant contaminants were observed, and the carotenoid peaks were ambiguous in the total ion chromatogram (SI-2, supporting information). Eleven major peaks at 450 nm UV–vis detection were observed (see Figure 2a), which had three maximum absorption wavelengths at 418, 441, and 468 nm in their PDA spectra (SI-3, supporting information), indicating that the 11 peaks were carotenoids and/or carotenoid esters in Figure 2a.6,8 The possible carotenoid diesters are summarized in SI-1 (supporting information), which were calculated with the combination between the carotenoid skeletons of lutein epoxide, violaxanthin, and neoxanthin and the fatty acids of laurate (C12:0), myristate (C14:0), palmitate (C16:0), and stearate (C18:0). The mass chromatograms were applied to the calculated exact masses of the carotenoid diesters as shown in Figures 2b–2d. Peaks 6 and 9, 7 and 10, and 8 and 11 were matched with the peaks of neoxanthin/violaxanthin diesters from their molecular weights of m/z 1021.8219, 1049.8532, and 1077.8845, respectively, in Figure 2. The PDA peaks 1–5 were matched with the neoxanthin/violaxanthin monoesters from their molecular weights of m/z 783.5922, 811.6235, and 867.6861 (SI-1, supporting information, and Figure 4). Neoxanthin and violaxanthin are isomers, and neoxanthin with three hydroxy groups is more hydrophilic than violaxanthin with two hydroxy groups. The neoxanthin ester was eluted earlier than the violaxanthin ester in their reversed-phase LC separation.8 Therefore, peaks 6, 7, and 8 were identified as the neoxanthin diesters, and peaks 9, 10, and 11 were identified as the violaxanthin diesters.

In their MS spectra, the dehydrated ions [M – H₂O + H]⁺ were observed with the protonated molecules [M + H]⁺ (Figure 3). Interestingly, the dehydrated ion peaks of neoxanthin esters predominated over the protonated molecule peaks, and the protonated molecule peaks of violaxanthin esters predominated under the same experimental conditions. There were differences in the dehydration generation tendencies between neoxanthin and violaxanthin esters in APCI. The mechanism of the dehydration differences is discussed later.

**FIGURE 5** CID MS/MS spectra of dehydrated neoxanthin/violaxanthin diesters at m/z 1003.8 at 21.5 min as peak 6 of dehydrated neoxanthin dimyristate (a), at m/z 1031.8 at 21.7 min as peak 7 of dehydrated neoxanthin myristate–palmitate (b), at m/z 1059.9 at 22.0 min as peak 8 of dehydrated neoxanthin dipalmitate/dehydrated neoxanthin myristate–stearate (c), at m/z 1003.8 at 22.3 min as peak 9 of dehydrated violaxanthin dimyristate (d), at m/z 1031.8 at 22.6 min as peak 10 of dehydrated violaxanthin myristate–palmitate (e), and at m/z 1059.9 at 22.8 min as peak 11 of dehydrated violaxanthin dipalmitate/dehydrated violaxanthin myristate–stearate (f)
CID MS/MS analysis was conducted to elucidate the fatty acid combinations in the neoxanthin/violaxanthin diesters (Figure 4). The fragment ions from the carotenoid esters were produced by the loss of the fatty acid parts in the CID MS/MS analysis because the ester bonds are weaker than C–C bonds. The CID MS/MS spectra of peaks 6 and 9 from the precursor ions at m/z 1021.8 (C₆₈H₁₀₉O₆) showed fragment ions generated by the loss of myristate (C₁₄:0) at m/z 793.7 (see Figures 4a and 4d). The CID MS/MS spectra of peaks 7 and 10 from the precursor ions at m/z 1049.8 (C₇₀H₁₁₃O₆) showed fragment ions generated by the loss of myristate (C₁₄:0) and palmitate (C₁₆:0) at m/z 793.7 and 821.7, respectively (Figures 4b and 4e). There were no fragment ions of the fatty acid combination of laurate and stearate. The CID MS/MS spectra of peaks 8 and 11 from the ions at m/z 1077.9 (C₇₂H₁₁₇O₆) showed an abundant fragment.

**Figure 6**  The cation structure and dehydration of neoxanthin diesters after protonation at the hydroxy group and the epoxide group (a) and those of violaxanthin diesters (b)
ion generated by the loss of palmitate at \( m/z \) 821.7 (Figures 4c and 4f). In addition to that, fragment ions at \( m/z \) 793.7 and 849.8 were observed in the MS/MS spectra, which corresponded to the loss of myristate and stearate, respectively. Therefore, both PDA peaks 8 and 11 \( (C_{72}H_{117}O_6) \) contained a major neoxanthin/violaxanthin dipalmitate and a minor neoxanthin/violaxanthin myristate–stearate (Figures 4c and 4f). Table 1 summarizes the elucidated neoxanthin/violaxanthin mono- and diester structures.

3.2 | Distinguishing between isomeric neoxanthin and violaxanthin esters from their in-source dehydrated fragment ions

In the MS spectra of peaks 6–11, peaks of dehydrated ions \([M - H_2O + H]^+\) were observed in addition to protonated molecules of carotenoid diesters (Figure 3).

We focused our attention on the in-source decay of dehydrated ions in the CID MS/MS spectra of the neoxanthin/violaxanthin esters as shown in Figure 5. The fragment ions resulting from the 92 \( u \) loss were observed from the dehydrated violaxanthin ester ions (Figures 5d–5f), but these fragment ions were not generated from the dehydrated neoxanthin esters (Figures 5a–5c). These fragments did not depend on the fatty acid species, and in the CID MS/MS spectra differences at 92 \( u \) loss product ions came from the structural differences between violaxanthin and neoxanthin. When the collision energies were changed from 15 to 45, the 92 \( u \) loss product ions were absent or weak in the CID MS/MS spectra of dehydrated neoxanthin esters (SI-5, supporting information). Conversely, the 92 \( u \) loss product ions were observed as abundant ions in the CID MS/MS spectra of dehydrated violaxanthin diesters.

3.3 | Dehydration mechanism of violaxanthin and neoxanthin diesters and their stable cation structures

Comparing the protonated molecules \([M + H]^+\) and the in-source decay dehydrated ions \([M - H_2O + H]^+\) in the LC/MS spectra of neoxanthin and violaxanthin diesters, the dehydrated ions \([M - H_2O + H]^+\) were predominant in neoxanthin diesters, and the protonated molecules \([M + H]^+\) were predominant in violaxanthin diesters (Figure 3). These tendencies can be explained by the ease with which the dehydration reaction occurs for both protonated carotenoid diesters, as shown in Figure 6.

The first step of dehydration is protonation at the oxygen atom of the hydroxy or epoxide group in neoxanthin diesters. As shown in Figure 6, the tertiary hydroxy group is easily dehydrated after protonation because of its location in the allyl position of the allene.
group. Conversely, the epoxide ring-opening reaction easily proceeds after protonation, but, at this stage, the dehydration reaction has not yet occurred. We estimate that after several steps, it may converge to the same allene allyl carbocation that is produced by the dehydration reaction of the tertiary hydroxy group. Although we speculate that the contribution of the dehydration reaction derived from the protonation of the epoxide group is lower than that of the tertiary hydroxy group, it is expected that the dehydration reaction proceeds easily in neoxanthin diesters (Figure 6).

The epoxide ring-opening reaction in violaxanthin diesters easily proceeds after protonation, but the dehydration step does not proceed smoothly because there is no factor to help the dehydration of the generated hydroxy group (Figure 6). Thus, we presume that protonated violaxanthin diesters are more stable than the dehydrated ones. Therefore, LC/MS showed that the dehydrated ion \([M - H_2O + H]^+\) was predominant in neoxanthin diesters, whereas the protonated molecule \([M + H]^+\) was predominant in violaxanthin diesters (Figure 3).

### 3.4 Proposal of the mechanism of 92 u loss fragmentation difference between the dehydrated neoxanthin and violaxanthin diesters

The 92 u loss product ions \([M - H_2O - 92u + H]^+\) were detected from the dehydrated violaxanthin diesters as the precursor ions \([M - H_2O + H]^+\), and they were not detected from the dehydrated neoxanthin diesters in their CID MS/MS spectra.
The 92 u loss product ions are one of the identical product ions for the carotenoid structure. The intramolecular \([2 + 2]\) cycloaddition proceeds at the carotenoid polyene structure, and then the 92 u species is removed from carotenoid molecules with the retro-[2 + 2] cycloaddition reaction. This 92 u loss mechanism has been reported previously.\(^{12,13}\) The 92 u loss product ions were detected in the CID MS/MS spectra of both free neoxanthin and violaxanthin. However, the 92 u loss product ions were either not detected or only in low abundance from the dehydrated precursor ions \([\text{M} - \text{H}_2\text{O} + \text{H}]^+\) of neoxanthin diesters in the CID MS/MS spectra.

The driving force of the \([2 + 2]\) cycloaddition of carotenoids is the depolarization of the appropriate double bond because of the stable carbocation that delocalized in the polyene structure of the carotenoid (see Figure 7). The carbocations of dehydrated violaxanthin diesters can be conjugated with and delocalize on the violaxanthin-polyene as shown in Figures 6 and 7. Because of the presence of this carbocation, the double bonds involved in the \([2 + 2]\) cycloaddition are polarized to \(\delta^+\) and \(\delta^–\), so that the \([2 + 2]\) cycloaddition reaction can easily proceed. The generated labile cyclobutane quickly opens, and a toluene species is removed through the retroreaction (Figure 7). Therefore, the 92 u loss product ions were preferentially generated from the dehydrated violaxanthin diesters.

Conversely, the dehydrated ions of neoxanthin diesters are the allene allyl carbocations that were generated from dehydration at the tertiary hydroxy group in Figure 6. The carbocation is not conjugated with the neoxanthin-polyene, and the double bonds in the neoxanthin-polyene are not effectively depolarized. Thus, the \([2 + 2]\) cycloaddition reaction does not proceed, and then the 92 u loss product ions are not generated from the dehydrated neoxanthin diesters (Figure 7).

3.5 Applications: Rapid identification of carotenoid pigment contained in yellow Mandevilla (Mandevilla sanderi) flowers

Mandevilla sanderi is a popular garden plant that has yellow flower petals. We used this flower to demonstrate the method for distinguishing the isomers of the neoxanthin/violaxanthin diesters. The LC/PDA chromatogram of the acetone extract from the Mandevilla flower showed five peaks of carotenoid esters (numbered 9’–13’ in Figure 8). The chemical composition formulas of the five peaks were elucidated from matching to the mass chromatogram of their combination of carotenoid skeletons and esters in SI-1 (supporting information). The structure of the \(\text{C}_{68}\text{H}_{109}\text{O}_6\) compound at peak 9’ was elucidated as the violaxanthin dimyristate ester.

The 92 u loss product ions were observed in the CID MS/MS from the dehydrated precursor ion of the \(\text{C}_{68}\text{H}_{109}\text{O}_6\) compound at peak 10’ in Figure 9b. Therefore, the compound eluting at peak 10’ was identified as violaxanthin myristate-palmitate. Table 2 elucidates and summarizes the structures of peak 9’–13’ compounds. Peak 11’ contained the three compounds, namely, violaxanthin dipalmitate, violaxanthin myristate-stearate, and lutein epoxide dimyristate esters. The carotenoid ester profiling of yellow petals was easily performed using the LC/PDA-APCI-MS.
and MS/MS from the molecule and dehydrated molecules including the identification of the isomeric neoxanthin/violaxanthin diesters.

| Peak no. | Retention time (min) | Precursor m/z | Product m/z [fragment ions (relative intensity)] | Compound |
|----------|---------------------|---------------|-----------------------------------------------|-----------|
| 9'       | 22.3                | 1021.8        | 1003.9 [M + H – 18]⁺ (10), 929.9 [M + H – 92]⁺ (5), 793.7 [M + H – 14:0]⁺ (100), 775.7 [M + H – 16:0]⁺ (10), 565.5 [M + H-14:0-14:0]⁺ (10) | Violaixin dimyristate |
| 10'      | 22.6                | 1049.9        | 1032.0 [M + H – 18]⁺ (20), 957.9 [M + H – 92]⁺ (5), 821.7 [M + H – 14:0]⁺ (100), 803.7 [M + H – 18:0]⁺ (10), 793.7 [M + H – 16:0]⁺ (85), 775.7 [M + H – 18-16:0]⁺ (10), 565.5 [M + H – 14:0-16:0]⁺ (10) | Violaixin myristate-palmiate |
| 11'-1    | 22.8                | 1077.9        | 1060.0 [M + H – 18]⁺ (20), 986.0 [M + H – 92]⁺ (5), 849.8 [M + H – 14:0]⁺ (20), 821.8 [M + H – 16:0]⁺ (100), 803.7 [M + H – 16-18:0]⁺ (10), 793.7 [M + H – 16:0]⁺ (15), 565.5 [M + H – 16-14:0]⁺ (10) | Violaixin dipalmitate and Violaixin myristate-stearate |
| 11'-2    | 22.8                | 1005.8        | 913.9 [M + H – 92]⁺ (20), 777.7 [M + H – 14:0]⁺ (100), 548.4 [M + H – 14-16:0]⁺ (10) | Lutein epoxide dimyristate |
| 12'      | 23.1                | 1033.9        | 941.9 [M + H – 92]⁺ (35), 805.8 [M + H – 14:0]⁺ (100), 777.7 [M + H – 16:0]⁺ (85), 548.4 [M + H – 16-14:0]⁺ (15) | Lutein epoxide myristate-palmitate |
| 13'      | 23.4                | 1061.9        | 969.9 [M + H – 92]⁺ (25), 805.8 [M + H – 16:0]⁺ (100), 548.4 [M + H – 16-16:0]⁺ (15) | Lutein epoxide dipalmitate |

Annotated peaks 9’-13’ are shown in Figure 6.

4 | CONCLUSIONS

The in-source dehydrated ions [M – H₂O + H]⁺ were useful for distinguishing neoxanthin/violaxanthin diesters. The dehydrated ions of neoxanthin diesters were predominant over their protonated molecules [M + H]⁺. The allene allyl carbocation in neoxanthin diesters was stable, which was generated by dehydration after preferential protonation at the hydroxy group. The protonated molecules of violaxanthin diesters were predominant. The epoxide group opened easily after protonation; however, the dehydration did not proceed at this stage.

The neoxanthin/violaxanthin diesters were identified by the 92 u loss product ions in the CID MS/MS spectra from the dehydrated molecules as a pseudoprecursor ion. The 92 u loss of C7H8 was explained by the intramolecular [2 + 2] cycloaddition, which proceeded preferably in dehydrated violaxanthin diesters because the carbocations in the dehydrated species were conjugated to the polyene and those double bonds were depolarized in CID MS/MS. Therefore, the isomeric neoxanthin/violaxanthin diesters
were distinguished using LC/PDA-APCI-MS and MS/MS. This method is practical and useful for the profiling of the carotenoid esters of the yellow petals.

PEER REVIEW
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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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