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The Identification of Vitamin E Homologues in Medicinal Plant Samples Using ESI(+)-LC-MS3

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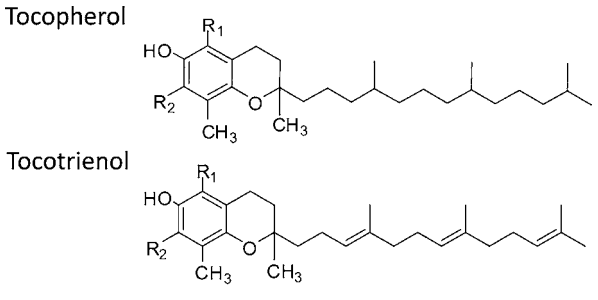
ABSTRACT: The aim of this study was to elucidate the presence of vitamin E homologues in medicinal plants. To identify various homologues in the matrix of medicinal plant samples, a method for simultaneous determination was developed using ESI(+)-LC-MS3. A complete separation of each homologue was achieved within 20 min using a PFP column and an isocratic elution system of water/methanol (10:90, v/v) at a flow rate of 0.5 mL/min. The ESI-MS condition for each homologue was optimized, and the *m/z* value and the fragmentation pathway of each homologue were summarized. This LC-MS3 method made it possible to detect the homologues without the effect of matrix; therefore, high sensitive analysis was established, and then, the MS3 makes it possible to extract from plants with methanol only. The LC-MS3 method was applied to identify the eight vitamin E homologues in 11 medicinal plants.

KEYWORDS: *ESI(+)-LC-MS3, simultaneous analysis, tocopherol, tocotrienol, fragmentation pattern, matrix effect*

INTRODUCTION

Vitamin E is a term used to designate a family of eight related fat-soluble homologues found in nature (*α*-, *β*-, *γ*-, and *δ*-tocopherols and *α*-, *β*-, *γ*-, and *δ*-tocotrienols), which share a common structure, as shown in Figure 1, with a chromanol head and phytyl tail.[1,2] The most well-known function of vitamin E is the chain-breaking antioxidant activity that prevents the cyclic propagation of lipid peroxidation; this protects body tissues against oxidative damage and helps to prevent cardiovascular diseases, neurological diseases, and cancer.[3,4] Although it is essential to sustain life, the human body cannot manufacture its own vitamin E, which must be provided by foods and supplements; therefore, it is an essential nutrient.[5] The most abundant sources of vitamin E are wheat germ oil, sunflower oil, canola/rapeseed oil, maize/corn oil, soybean oil, palm oil, and olive oil. Nuts, sunflower seeds, sea buckthorn berries, and wheat germ are also good sources. Other sources of vitamin E are whole grains, fishes, peanut butter, and green leafy vegetables.[5]



Tocopherol / Tocotrienol	R1	R2
α	CH3	CH3
β	CH3	H
γ	H	CH3
δ	H	H

Figure 1. Chemical structures of tocopherol and tocotrienol.

Several studies have been performed to develop sensitive and selective methods for determining tocopherols and tocotrienols in animals,[6−8] foods,[9−15] and plants.[16,17] A normal-phase high-performance liquid chromatographic (NP-HPLC) method with a fluorescence detector has been developed for simultaneous quantification of eight vitamin E homologues in rice, rice bran, rice, plants, and rats;[7,9,11,12] however, the limit of detection (LOD) of this method is 50 ppb.[9] On the other hand, a reverse-phase high-performance liquid chromatographic (RP-HPLC) method with a fluorescence detector has been developed for simultaneous quantification of eight vitamin E homologues in cereals. However, the LOD of this method is 750 ppb,[13] but these methods cannot separate the vitamin E from the substances that elute at the same retention time and have fluorescence. Furthermore, complex treatment, especially cumbersome saponification treatment, was necessary to measure the vitamin E homologues in samples with these methods.[4,12] On the contrary, a simple methanol extraction process may be applied to simultaneous analysis of vitamin E homologues in our study. Therefore, a more sensitive method with mass spectrometry detection was developed. Negative ion atmospheric pressure chemical ionization (APCI) has therefore been chosen to develop the liquid chromatography−mass spectrometry

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Table 1. MS Operation Parameters[a]

	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	α-tocotrienol	β-tocotrienol	γ-tocotrienol	δ-tocotrienol
				MS2				
m/z	431 → 165	417 → 151	417 → 151	403 → 137	425 → 165	411 → 151	411 → 151	397 → 137
scan rate (Da/s)	10000	10000	10000	10000	10000	10000	10000	10000
LIT fill time (ms)	1	1	1	1	1	1	1	1
				MS3				
m/z	431→165→137	417→151→123	417→151→123	403→137→109	425→165→137	411→151→123	411→151→123	397→137→109
scan rate (Da/s)	1000	1000	1000	1000	1000	1000	1000	1000
LIT fill time (ms)	10	10	10	10	10	10	10	20
excitation time (ms)	20	20	20	20	20	20	20	20
AF2	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.05
				MS2 and MS3				
DP (V)	56	61	55	76	71	61	121	81
CE (V)	39	37	45	35	37	35	35	37

[a]DP, declustering potential; CE, collision energy; and AF2, auxiliary RF (radio frequency) 2.

(LC-MS) method for simultaneous determination. A rapid and sensitive method for the simultaneous quantification of α-tocopherol and four carotenoids in botanical materials has been developed using NP-LC-APCI-tandem mass spectrometry with 35 ppb limit of quantitation (LOQ) but measured only α-tocopherol.[15] The four tocopherols (α-, β-, γ-, and δ-tocopherols) in foods (sunflower and milk) have been analyzed by APCI and an electropsray ionization (ESI) method with 3 ppb LOD, but tocotrienols were not analyzed.[4] The six vitamin E components (α-, β-, γ-, and δ-tocopherols and β- and γ-tocotrienols) in rice bran and germ have been analyzed by liquid chromatography−mass spectrometry/mass spectrometry (LC-MS2) with ESI mode;[10] however, the LOD was not shown, and α- and δ-tocotrienol were not found in the figure. In another study, α-tocopherol was found in rosemary using HPLC in combination with photodiode array detection; however, homologues other than α-tocopherol were not analyzed in this report.[16]

As described above, there is little information about the eight homologues of vitamin E in plants. We focused on developing a simple and sensitive analysis method for four tocopherols and four tocotrienols. LC-MS3 has recently been used in pharmaceutical, biochemical, and food chemical fields as a rapid strategy for sensitive analyses to analyze the other chemicals.[18−24] Therefore, the LC-MS3 method was applied in this present study to measure the four tocopherols and four tocotrienols in plants. This LC-MS3 method enabled the simultaneous determination of vitamin E homologues without the effect of the matrix from samples as compared to LC-MS2. This analysis method is highly sensitive with a simple extraction procedure.

MATERIALS AND METHODS

Chemicals and Reagents. α-, β-, γ-, and δ-Tocopherols (the purity was >98.5%) and α-, β-, γ-, and δ-tocotrienols (the purity was >98.5%) were gift from Eizai Co. (Tokyo, Japan) and Eizai Food Chemical Co. (Tokyo, Japan). Methanol of HPLC grade was purchased from Wako Pure Chemical Industries (Osaka, Japan), and water was purified by a Milli-Q integral purification system (Millipore, Bedford, MA).

Each tocopherol and tocotrienol standard (2 mg) was dissolved in 20 mL of methanol to prepare a stock solution (100 μg/mL), which was stored in brown bottles. A 2 mL amount of the obtained stock solution was diluted in methanol to 20 mL to use as a separation working solution (10 μg/mL) and stored at below 4 °C.

HPLC-ESI/MS Conditions. LC experiments were conducted with a 1200 series LC system (Agilent Technologies, Santa Clara, CA) comprising a binary pump, degasser, autosampler, and column oven. Chromatographic separation was achieved in a PFP column (150 mm × 4.6 mm; 5 μm particle size; 300 Å pore size; Thermo Fisher Scientific Fluophase, Runcorn, Cheshire, England) at 40 °C with an injection volume of 5 μL. The mobile phase was delivered at 0.5 mL/min consisting of water as solvent A and methanol as solvent B. Binary isocratic elution was performed with 10% A to 90% B.

A QTRAP5500 mass spectrometer (AB SCIEX, Foster City, CA) equipped with an ESI source in the positive ion mode was used under the following operating conditions: 10 psi CUR, 5500 V ion spray voltage, 700 °C temperature, 70 psi GS1, 70 psi GS2, CAD medium, and 10 V EP. Table 1 outlines the values set for each tocopherol and tocotrienol studied.

Plant Material. Eleven kinds of unprocessed medicinal plants (*Ocimum basilicum*, *Stevia rebaudiana*, *Rubus idaeus*, *Mentha spicata*, *Mentha arvensis* var. *piperascens*, *Melissa officinalis*, *Eucalyptus maculate*, *Eucalyptus citriodora*, *Eucalyptus robusta* Sm., *Hypericum perforatum*, and *Foeniculum vulgare*) were obtained from the Medical Plant Garden of Showa Pharmaceutical University (Tokyo, Japan). The plants used were collected, identified, and grown by Takahisa Nakane and Akihito Takano, etc., plant scientists at the garden.

Sample Preparation. The leaves of these medicinal plants were freeze-dried and then crushed. A 0.2 g amount of the crushed dehydrated leaves was extracted overnight three times with 2 mL of methanol at room temperature for analytical purposes. The methanolic extracts were combined and stored at below −80 °C.

Method Validation. To examine whether each vitamin E homologue in plant extracts could be precisely identified, we investigated the effects of matrix derived from plant extracts using LC-MS2 and LC-MS3. Briefly, 0.1 mL of the standard mixture containing each vitamin E homologue (5000 μg/mL) was spiked into 0.9 mL of each plant extract. This solution was analyzed by LC-MS2 and LC-MS3. The recovery was calculated in accordance with the formula: Rec (%) = $\{[C(a) - C(b)]/C(c)\} \times 100$, where Rec was the recovery, $C(a)$ was the concentration in spiked sample, $C(b)$ was the initial concentration, and $C(c)$ was the concentration of standard mixture of tocopherols and tocotrienols. The experiment was done in triplicate. Values were expressed as means ± SDs.

RESULTS AND DISCUSSION

Ionization Patterns of the Tocopherols and Tocotrienols. The ESI-MS conditions were optimized by injecting methanol solutions of the standard α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols (10 μg/mL) directly. The results of MS3 analyses are shown in Figure 2; only MS2 data of

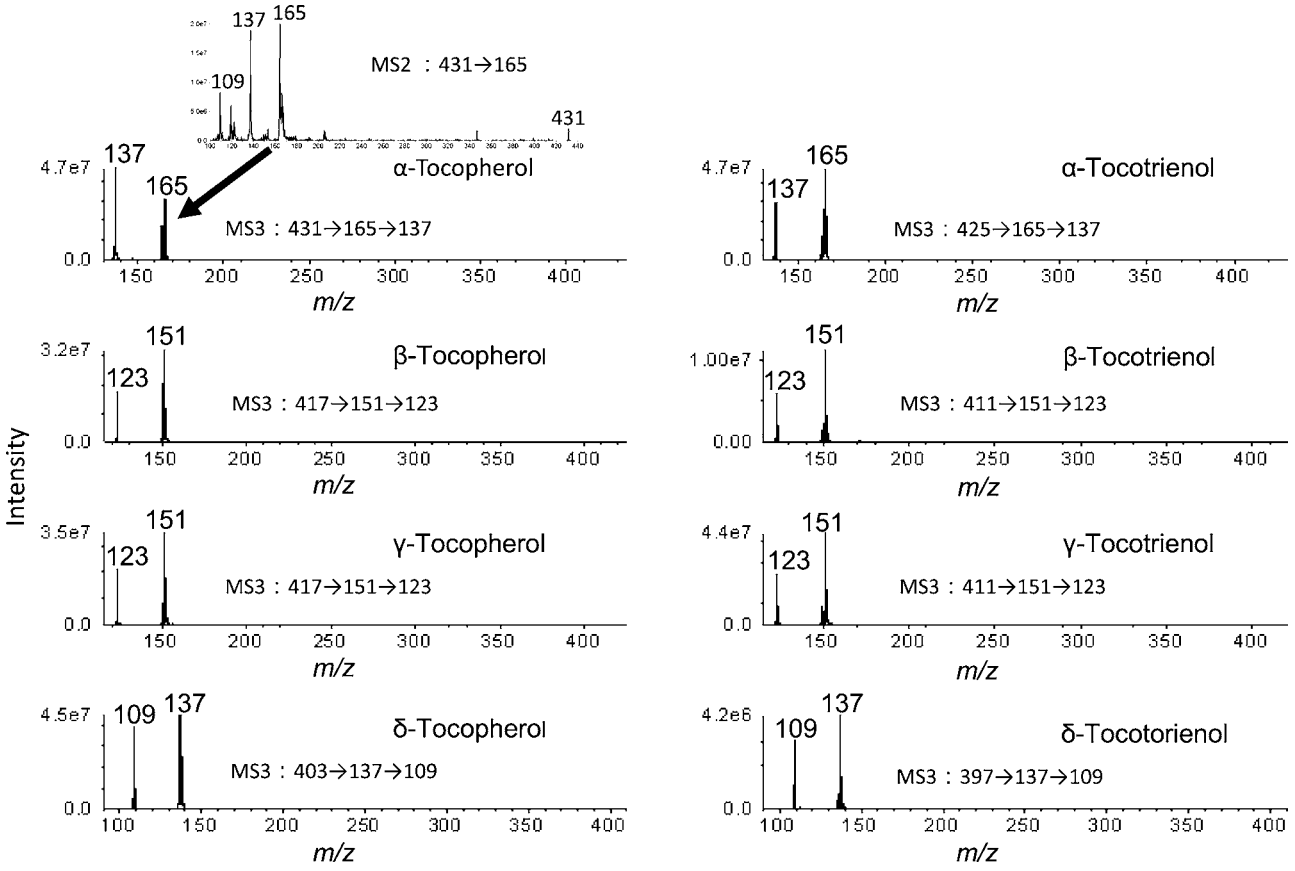

Figure 2. Positive ESI-MS3 data of *α*-, *β*-, *γ*-, and *δ*-tocopherols and *α*-, *β*-, *γ*-, and *δ*-tocotrienols with ESI-MS2 data of *α*-tocopherol. Each standard of tocopherols and tocotrienols was injected to mass spectrometer (QTRAP5500) directly. The MS3 spectrum of eight homologues was shown, and the MS2 spectrum of *α*-tocopherol was also shown. Fragmentation procedures are shown in Table 1.

α-tocopherol were shown in Figure 2; from those data, the MS3 condition for each homologue was optimized, and the *m*/*z* value of optimized MS3 conditions was summarized in Table 1.

The fragmentation pathway for the $[M + H]^+$ ion of *α*-tocopherol at *m*/*z* 431 is shown in Figure 3a. The $[M + H]^+$ ion gave an intense signal at *m*/*z* 165 [Frag1 + H in Figure 3a]$^+$, which is fragmentation peak from *m*/*z* 431, the same as described in Figure 3a.[6,10] Further fragmentation of this ion was verified by MS3, showing an intense signal at *m*/*z* 137 [Frag2 + H in Figure 3a]$^+$. The fragment data of *α*-tocotrienol were *m*/*z* 425 → *m*/*z* 165 → *m*/*z* 137 (Table 1), which was fragment 1 and fragment 2 in Figure 3b. The same mechanism is suggested for the formation of respective marker ions *m*/*z* 151 and *m*/*z* 123 for the *β*-, *γ*-, and *δ*-homologues (Figures 4a–d) as suggested by Kornél and Shanggong.[6,10]

The *β*- and *γ*-tocopherols gave the $[M + H]^+$ ion at *m*/*z* 417 in the full-scan mass spectrum (Table 1). The MS2 data for *m*/*z* 417 revealed [Frag1 + H in Figure 4a]$^+$ at *m*/*z* 151 as the base peak, resulting from the loss of 266 Da. A characteristic ion of [Frag2 + H in Figure 4a]$^+$, *m*/*z* 123, was one of the principal peaks in the MS3 data for *m*/*z* 151 (Figure 4a,b).

The *β*- and *γ*-tocotrienols gave the $[M + H]^+$ ion at *m*/*z* 411 in the full-scan mass spectrum (Table 1). The MS2 data for *m*/*z* 411 revealed [Frag1 + H in Figure 4c]$^+$ at *m*/*z* 151 as the base peak, resulting from the loss of 260 Da. A characteristic ion of [Frag2 + H in Figure 4c]$^+$, *m*/*z* 123, was one of the principal peaks in the MS3 data for *m*/*z* 151 (Figure 4c,d). These results showed that the fragmentation pattern could not be employed in the discrimination between *β*- and *γ*-tocopherol and between

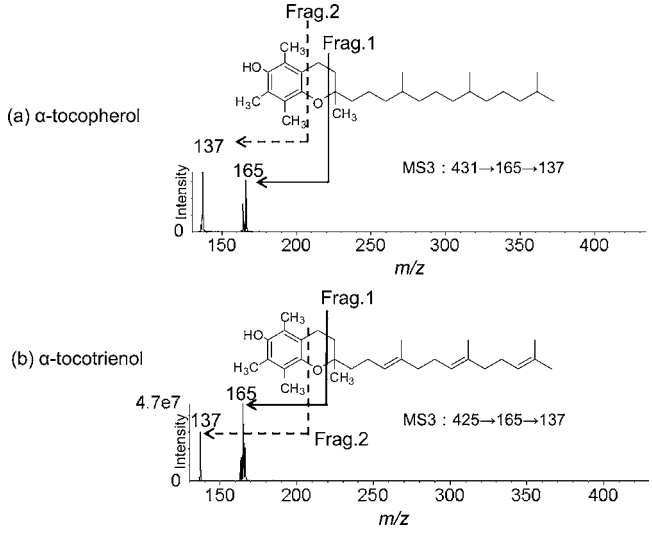

Figure 3. Proposed fragmentation patterns for (a) *α*-tocopherol and (b) *α*-tocotrienol. (a) The main peak of *α*-tocopherol was fragmentated to *m*/*z* 165 and then further fragmentated. The mass spectrum of MS3 was shown at the bottom. (b) The main peak of *α*-tocotrienol was fragmentated to *m*/*z* 165 and then further fragmentated. The mass spectrum of MS3 was shown at the bottom.

β- and *γ*-tocotrienol, which have the same number of methyl groups with different positions in the chroman ring. It was found that *m*/*z* 123 was one of the principal peaks in the MS3 data for *β*- and *γ*-tocopherol and for *β*- and *γ*-tocotrienol.

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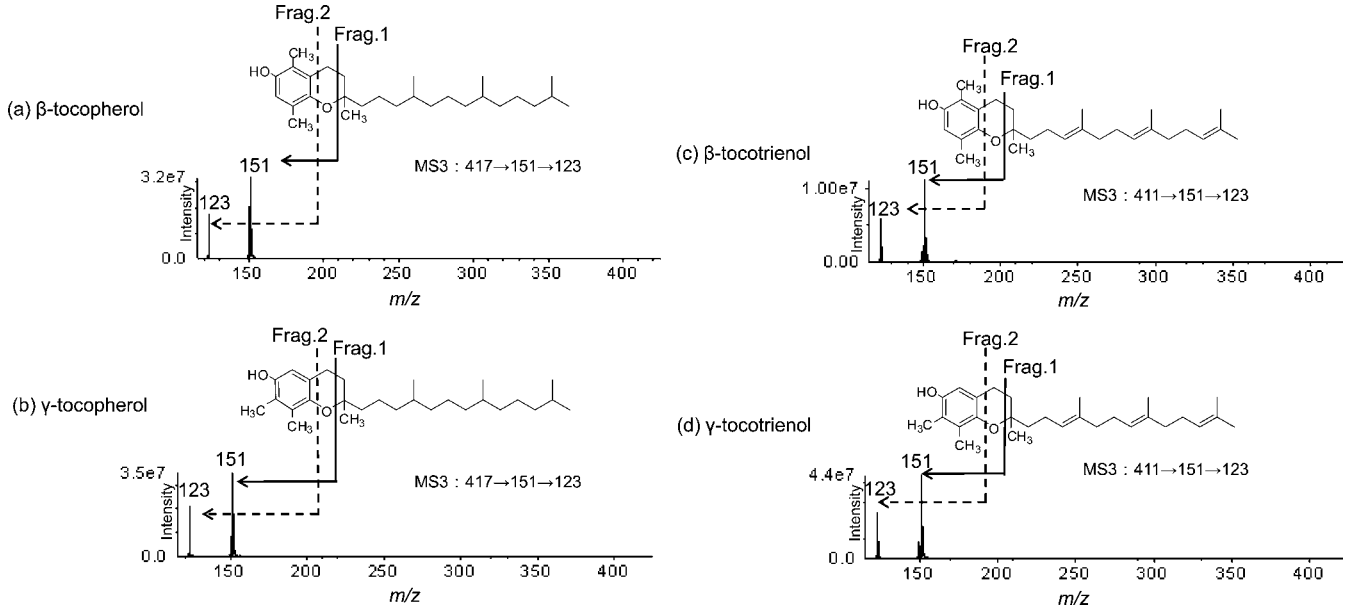

Figure 4. Proposed fragmentation patterns for (a) β-tocopherol, (b) γ-tocopherol, (c) β-tocotrienol, and (d) γ-tocotrienol. (a) The main peak of β-tocopherol was fragmentated to m/z 151 and then further fragmentated. The mass spectrum of MS3 is shown at the bottom. (b) The main peak of γ-tocopherol was fragmentated to m/z 151 and then further fragmentated. The mass spectrum of MS3 is shown at the bottom. (c) The main peak of β-tocotrienol was fragmentated to m/z 151 and then further fragmentated. The mass spectrum of MS3 is shown at the bottom. (d) The main peak of γ-tocotrienol was fragmentated to m/z 151 and then further fragmentated. The mass spectrum of MS3 is shown at the bottom.

The $[M + H]^+$ ion at m/z 403 of δ-tocopherol was fragmented into the [Frag1 + H in Figure 5a]$^+$ ion at m/z 137 in the MS2 and then, the product [Frag2 + H in Figure 5b]$^+$ ion at m/z 109 was observed in the MS3 experiment (Figure 5b). These data are the first report to reveal the MS3 fragmentation pattern of the vitamin E homologues (four tocopherols and four tocotrienols).

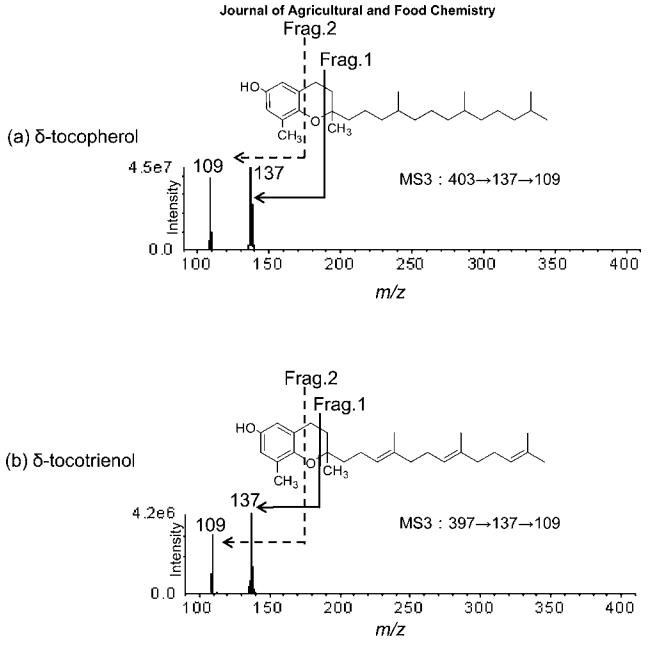

Figure 5. Proposed fragmentation patterns for (a) δ-tocopherol and (b) δ-tocotrienol. (a) The main peak of δ-tocopherol was fragmentated to m/z 137 and then further fragmentated. The mass spectrum of MS3 is shown at the bottom. (b) The main peak of δ-tocotrienol was fragmentated to m/z 137 and then further fragmentated. The mass spectrum of MS3 is shown at the bottom.

HPLC Separation and Simultaneous Determination of the Vitamin E Homologues. The LC-MS3 method for simultaneous determination of all vitamin E homologues (tocopherols and tocotrienols) with reversed-phase HPLC was developed (Figure 6). Reversed-phase HPLC was applied in the present study for the application of ESI-MS method. The elution order of the vitamin E components has been reported to be β- and γ-tocotrienols and δ-, β-, γ-, and α-tocopherols in reverse phase.[4,10] The fragmentation pattern in the MS3 data did not discriminate between the β- and the γ-tocopherols and

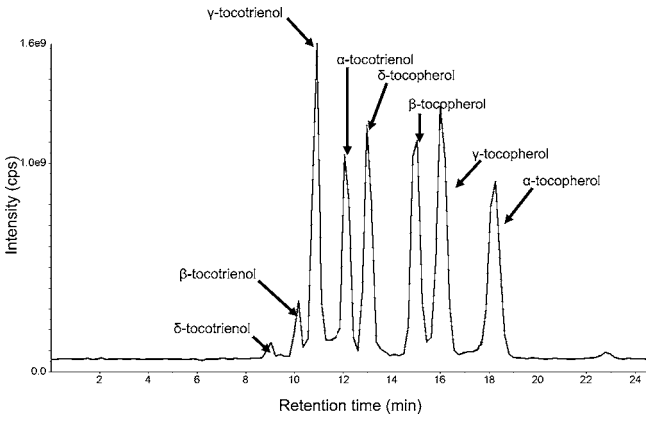

Figure 6. Chromatogram for separation of the four tocopherols and four tocotrienols using LC-MS3. MeOH:H_2O (90:10, v/v) as the mobile phase, T = 35 °C, and a 0.5 mL/min flow rate were used. The MS3 analysis process was set for the measurement of eight vitamin E homologues from 8 to 20 min.

data. The MS3 spectrum showed that the [Frag1 + H in Figure 5a]$^+$ ion yielded the fragment [Frag2 + H in Figure 5a]$^+$ ion at m/z 109.

The $[M + H]^+$ ion at m/z 397 of δ-tocotrienol produced the [Frag1 + H in Figure 5b]$^+$ ion at m/z 137 in the MS2 spectrum,

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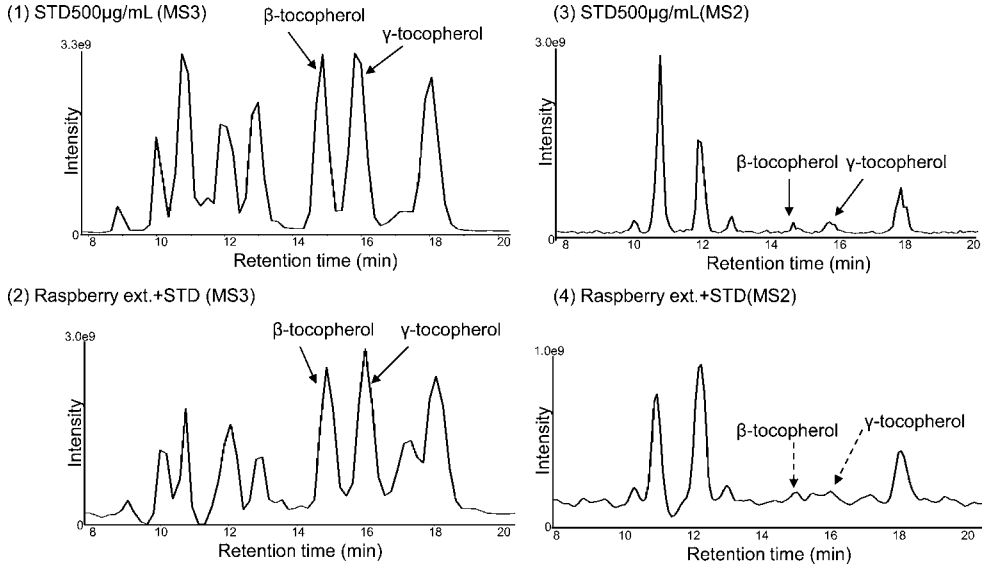

Figure 7. Effect of components of the raspberry extract on the determination of tocopherols and tocotrienols by LC-MS2 and LC-MS3. The standard mixture of eight vitamin E homologues in panel 1 was analyzed using LC-MS3 method (Table 1, MS3 parameters). The standard mixture of vitamin E homologues in panel 3 was analyzed using LC-MS2 method (Table 1, MS2 parameters). The raspberry extract (0.9 mL) was spiked with a standard solution of the vitamin E homologues (5000 μg/mL in 0.1 mL, panels 2 and 4).

tocotrienols because those had the same number of methyl groups at different positions in the chroman ring (Figure 1). When the NP-HPLC was used, relatively easy separations of homologues β- and γ-tocopherols and tocotrienols were obtained;[1,9,11,12,14] however, the NP-LC method is poor reproducibility and low stability.[1] On the contrary, under RP-HPLC using ODS column, β- and γ-homologues have not been separated.[1,14] Although these homologues can be separated under RP-HPLC conditions using long chain alkyl-bonded C30 silica, four tocotrienols have not been analyzed.[13] However, the new method using LC-MS3 with PFP column led to the complete separation of these compounds and rapid determination within 20 min (Figure 6). In this LC-MS3 analytical method, the LOD of the eight vitamin E homologues was 5 ppb, and it had about 10 times higher detection sensitivity as compared to previous analysis methods.[9,14] The MS response of δ-tocotrienol showed the lowest sensitivity among the vitamin E homologues. It is suggested that this phenomenon was attributable to a hydroxyl group (electron-withdrawing group) at the 5- and 7-positions in δ-tocotrienol, which may difficult to attach a proton during ionization process. It is concluded that the vitamin E homologues could be simultaneously determined using this LC-MS3 method.

Verification of the Usefulness of LC-MS3 for Confirming the Vitamin E Homologues and Matrix Effect in Medicinal Plant Extract. To investigate the effect of matrix from raspberry (Figure 7) and spearmint (Figure 8), eight homologues were added to these extracts and analyzed by LC-MS2 and LC-MS3. The sensitivity of the MS signal of vitamin E homologues in the raspberry and spearmint extracts was decreased in LC-MS2; however, it was not changed in LC-MS3; even the extract was added. Especially the LC-MS2 sensitivity of the β- and γ-tocopherols was decreased in the presence of the raspberry and spearmint matrix, and it was not possible to detect (Figures 7 and 8). The recoveries of the two MS procedures were summarized in Table 2. Recoveries were 10−63% for MS2 and 41−97% for MS3. The relative standard deviation (n = 3) for MS2 was below 25%, and for MS3, it was below 18%, showing that MS3 is the most precise procedure followed by MS2. The LOD was 5 ppb for this LC-MS3, rather sensitive as compared to previous methods.[9,14] According to the MS3 results, it was possible to detect the vitamin E homologues without the effect of matrix, we concluded that this LC-MS3 method was superior to previous reports,[9,14] and this is the first report to confirm the eight vitamin E homologues without any effect of matrix. The HPLC with fluorescence may detect the fluorescence from the matrix; therefore, LC-MS3 is a better method to analyze the vitamin E homologues in natural materials. Furthermore, this MS3 needs only a methanol extraction process without saponification of the sample. Furthermore, the LOD of this method was 10 times higher than previous methods.[9,14]

Identification of the Vitamin E Homologues in Medicinal Plants by ESI(+)-LC-MS3. The α-tocopherol has been detected in the leaves of *Rosmarinus officinalis* by HPLC in combination with photodiode array detection.[16] The rosemary seed extract has been found to contain α-, β-, and γ-tocopherols and α-, β-, and γ-tocotrienols by HPLC in combination with fluorescence detection.[17] However, except basil, spearmint, and peppermint, the presence of vitamin E in other medicinal plants has not previously been investigated.[25−28] Therefore, the LC-MS3 method was applied to determine tocopherols and tocotrienols in 11 kinds of medicinal plant. Table 3 shows the presence of vitamin E homologues in the medicinal plant extracts. α-Tocopherol was detected in all extracts of the 11 medicinal plants samples tested. α-Tocopherol is the most active antioxidant among the vitamin E homologues, and it is suggested that all of these medicinal plants have antioxidative activity. Although Coronado et al.[29] did not detect β-tocopherol in basil and spearmint by HPLC (fluorescence), our study confirmed the presence of β-tocopherol in basil and spearmint using a LC-MS3 technique. The β-tocopherol was also detected in three types of eucalyptus. The presence of γ-tocopherol was shown in basil, spearmint, raspberry, lemon balm, and fennel. Moreover, δ-tocopherol was identified in raspberry and spearmint (Figure 9a,b). On the other hand, our study has revealed that among the tocotrienols, only δ-tocotrienol was detected in

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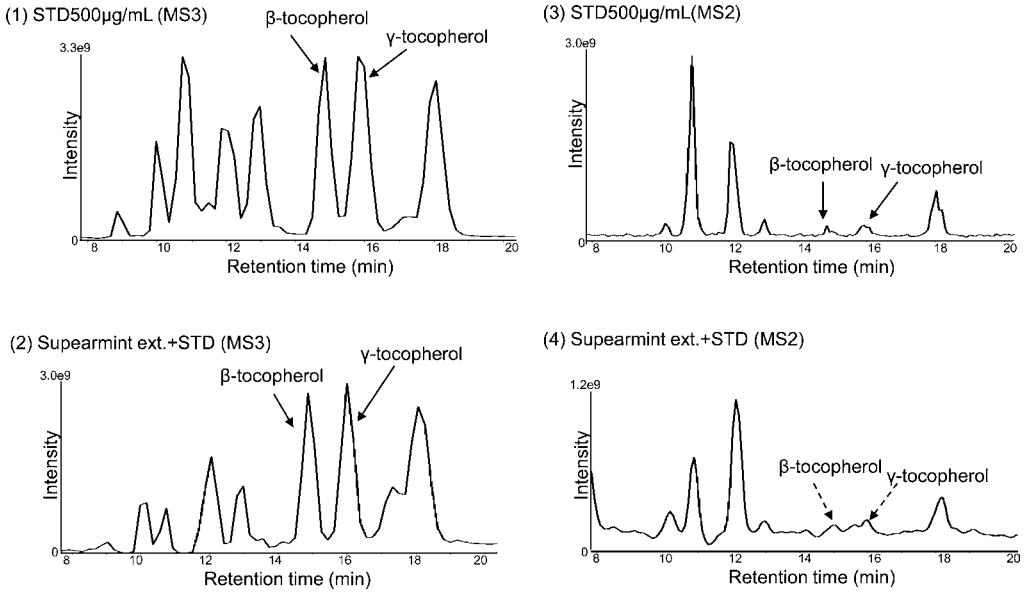

Figure 8. Effect of components of the spearmint extract on the determination of tocopherols and tocotrienols by LC-MS2 and LC-MS3. The standard mixture of eight vitamin E homologues in panel 1 was analyzed using LC-MS3 method (Table 1, MS3 parameters). The standard mixture of vitamin E homologues in panel 3 was analyzed using LC-MS2 method (Table 1, MS2 parameters). The spearmint extract (0.9 mL) was spiked with a standard solution of the vitamin E homologues (5000 μg/mL in 0.1 mL, panels 2 and 4).

Table 2. Recovery of Vitamin E from Raspberry and Spearrmint by LC-MS2 and LC-MS3[a]

	recovery (%)			
	raspberry		spearmint	
compds	MS2	MS3	MS2	MS3
α-tocopherol	47.46 ± 18.63	97.74 ± 10.84	24.22 ± 5.13	87.28 ± 8.18
β-tocopherol	38.22 ± 8.93	86.14 ± 9.79	58.77 ± 10.42	94.49 ± 8.72
γ-tocopherol	26.52 ± 10.47	83.27 ± 14.64	48.91 ± 6.74	79.68 ± 8.91
δ-tocopherol	34.56 ± 6.75	70.97 ± 18.95	36.52 ± 5.34	67.73 ± 12.66
α-tocotrienol	63.87 ± 12.72	76.61 ± 11.10	41.91 ± 15.78	93.34 ± 6.84
β-tocotrienol	32.78 ± 25.66	69.57 ± 8.48	58.68 ± 12.14	88.75 ± 8.81
γ-tocotrienol	33.08 ± 6.72	68.38 ± 13.44	10.96 ± 8.13	41.16 ± 8.85
δ-tocotrienol	21.03 ± 9.22	92.50 ± 5.22	60.36 ± 10.16	66.44 ± 13.50

[a] n = 3, average (%) ± SD.

Table 3. Plant Species Containing Tocopherols and Tocotrienols Are Listed[a]

		compds							
		tocopherol				tocotrienol			
common name	species	α	β	γ	δ	α	β	γ	δ
basil	*Ocimum basilicum*	+	+	+	−	−	−	−	−
sweetleaf	*Stevia rebaudiana*	+	−	−	−	−	−	−	−
raspberry	*Rubus idaeus*	+	−	+	+	−	−	−	−
spearmint	*Mentha spicata*	+	+	+	+	−	−	−	−
Japanese peppermint	*Mentha arvensis var. piperascens*	+	−	−	−	−	−	−	−
lemon balm	*Melissa officinalis*	+	−	+	−	−	−	−	−
spotted gum	*Eucalyptus maculata*	+	+	−	−	−	−	−	−
lemon eucalyptus	*Eucalyptus citriodora*	+	+	−	−	−	−	−	−
swamp mahogany	*Eucalyptus robusta Sm.*	+	+	−	−	−	−	−	−
St. John's wort	*Hypericum perforatum*	+	−	−	−	−	−	−	+
fennel	*Foeniculum vulgare*	+	−	+	−	−	−	−	−

[a] +, present; −, detectable. LOD, 5 ppb.

St. John's Wort (Figure 9c), and α-, β-, and γ-tocotrienols were not detected in any of the 11 kinds of medicinal plants. The identification of δ-tocotrienol in St. John's Wort was interesting because it is first report that δ-tocotrienol was detected in leaf.[30]

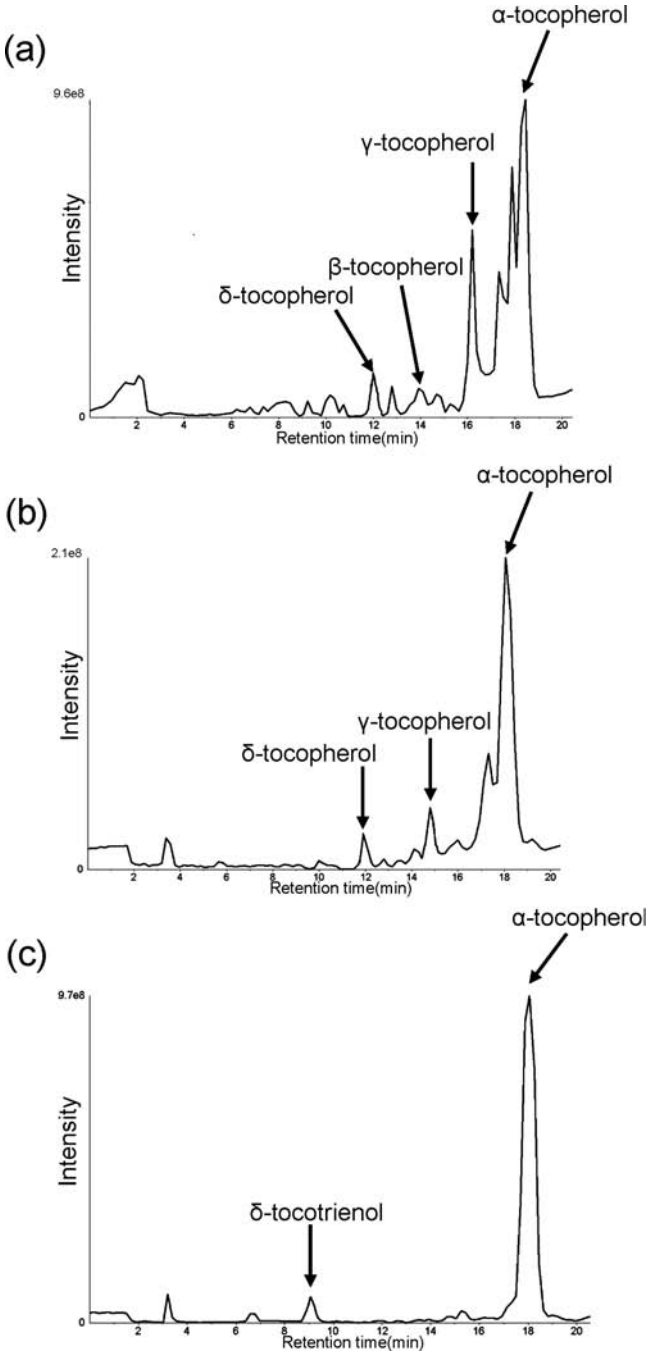

Figure 9. Chromatograms of natural plants. Chromatograms of (a) spearmint extract, (b) raspberry extract, and (c) St. John's Wort extract using LC-MS3.

This is first report to reveal the MS3 fragmentation pattern of the vitamin E homologues (four tocopherols and four tocotrienols) and developed a new method for the simultaneous determination of vitamin E homologues by ESI-(+)LC-MS3. This LC-MS3 method has about 10 times higher detection sensitivity as compared to previous analyses. Furthermore, the LC-MS3 technique makes it possible to extract simply with methanol and confirm vitamin E homologues without matrix effects as compared to the LC-MS2 technique. Using LC-MS3, we have revealed the presences of vitamin E homologues in many medicinal plants, and it was interesting that St. John's Wort contained γ-tocotrienol. In this study, we have successfully developed a sensitive, efficient, and reliable LC-MS3 method for eight vitamin E homologues in plants. This LC-MS3 would be applied to another field.

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Notes

The authors declare no competing financial interest.

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