

Tocopherols

We found that tocopherol, a fat-soluble vitamin, is less polar and less sensitive in ESI. Despite this, in our L3047 application, we successfully detected tocopherol in FIA-MS by using an ammonia eluent. Additionally, we compared the detection sensitivity of tocopherol in LC-MS/MS analysis with different types of eluents, and also compared ionization using APCI, commonly employed for low-polarity component mass spectrometry, and ESI.

Key words : Fat-soluble vitamin vitamin E tocopherol LC-MS/MS

Column : USP category: L1

[Analytical conditions]

Column : L-column3 C18 (ODS, 2 μ m, 12 nm); 2.1 mm I.D. \times 50 mm L.; Cat. No. 813140

Eluent : A: Aqueous eluent; B: 2-Propanol/CH₃CN (50/50)
A/B (20/80)

Flow rate : 0.4 mL/min

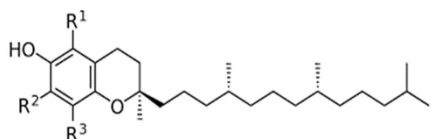
Temperature : 40 $^{\circ}$ C

Detection : ESI-MS/MS (-)

Injection volume : 1 μ L

System : LC: Ultimate 3000 Bio RS (Thermo Fisher Scientific K.K.); MS/MS: 3200 QTRAP (SCIEX)

Sample : Tocopherols, Vitamin E



	R ¹	R ²	m/z
α -Tocopherol	CH ₃	CH ₃	429.3/163.0
β -Tocopherol	CH ₃	H	415.5/149.1
γ -Tocopherol	H	CH ₃	415.5/149.1
σ -Tocopherol	H	H	401.5/135.1

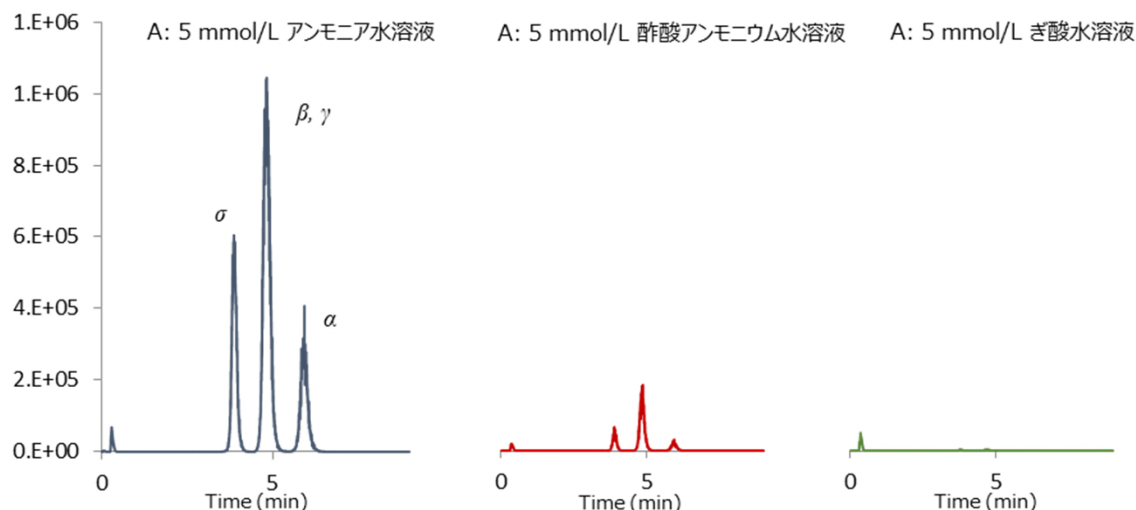


Fig.1 Comparison of eluent type and detection sensitivity

Tocopherol, a fat-soluble vitamin, is known to be less polar and less sensitive to mass spectrometry when using conventional ESI. However, our study has shown that the addition of ammonia to the eluent can significantly improve detection sensitivity. This effect is attributed to the increased pH of the eluent, which dissociates the phenol groups and enhances ionization of the tocopherol molecule.

we compared the detection sensitivity of Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) techniques for HPLC analysis. The analysis was carried out in positive mode for APCI, using formic acid as the eluent, and in negative mode for ESI, using ammonia as the eluent.

[Analytical conditions]

Column : L-column3 C18 (ODS, 2 μ m, 12 nm); 2.1 mm I.D. \times 50 mm L.; Cat. No. 813140
 Eluent : ESI A: 0.1% NH₃ in H₂O ; B: 2-Propanol/CH₃CN (50/50)
 APCI A: 0.1% HCOOH in H₂O ; B: 2-Propanol/CH₃OH (50/50)
 A/B (20/80) \rightarrow (1/99) 0 min \rightarrow 5 min
 Flow rate : 0.4 mL/min
 Temperature : 40 $^{\circ}$ C
 Detection : ESI-MS/MS(-) or APCI-MS/MS(+)
 Injection volume : 1 μ L
 System : LC: Ultimate 3000 Bio RS (Thermo Fisher Scientific K.K.); MS/MS: 3200 QTRAP (SCIEX)
 Sample : 1 mg/L in CH₃CN (each)

	R ¹	R ²	ESI-MS/MS(-) m/z	APCI-MS/MS(+) m/z
	CH ₃	CH ₃	429.3/163.0	431.2/165.2
	CH ₃	H	415.5/149.1	417.2/151.1
	H	CH ₃	415.5/149.1	417.2/151.1
	H	H	401.5/135.1	403.2/129.2

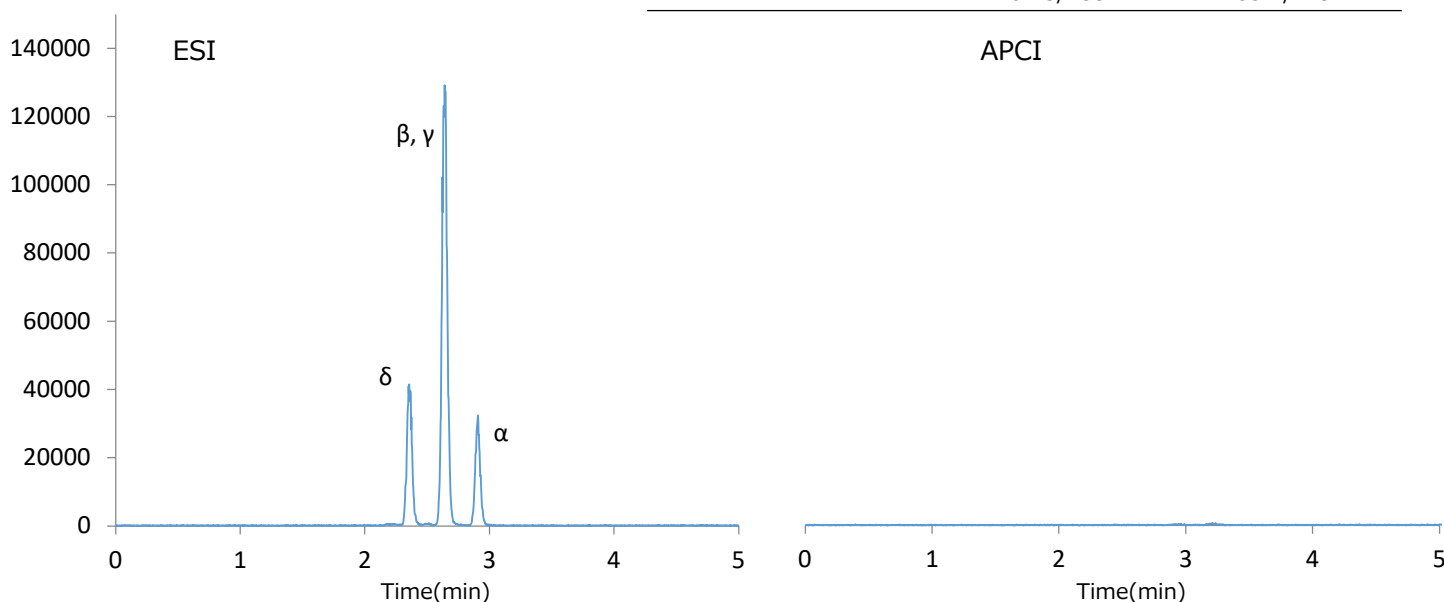


Fig.2 Comparison of detection sensitivity in ESI and APCI

By using an ammonia eluent, we achieved high sensitivity in the analysis of low-polarity tocopherols via ESI. Detection sensitivity increased with higher concentrations of ammonia in the eluent. Despite the extended use of the ammonia eluent to collect the data, L-column 3, which is highly durable, was still usable without requiring column replacement.

However, it is important to note that ammonia eluent can be corrosive to glass containers and metal pipes. Its reaction with CO₂ in the air may also result in changes in pH. Therefore, we recommend replacing the ammonia eluent within 1-2 days after use. If the system is not utilized for a prolonged period, it is also advisable to replace the ammonia solution with a solvent such as water/methanol or water/acetonitrile.