Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 241–246

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Reaction monitoring of tocopherols with active nitrogen oxides by ultra high-speed liquid chromatography

Yoshiko Nagata, Tadashi Nishio, Hideko Kanazawa*

Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan

ARTICLE INFO

Article history:
Received 9 October 2010
Received in revised form
21 December 2010
Accepted 24 December 2010
Available online 13 January 2011

Keywords:
Ultra high-speed liquid chromatography
Tocopherol
Active nitrogen oxide
Reaction monitoring

ABSTRACT

Ultra high-speed liquid chromatography (LC) has become increasingly popular in analytical research fields. This analytical system provides fast and efficient chromatographic separation over a wide range of flow rate and pressure. In this study, we applied an ultra high-speed LC system to monitor the reaction of α-, γ-, and δ-tocopherols with active nitrogen species. By using an ultra high-speed LC system equipped with a photo-diode array detector, short time analysis and detection of a wide range of reaction products were accomplished efficiently. The analysis time of tocopherol and its major oxidation products were greatly shortened compared to conventional HPLC methods (more than 10 times). The ultra reversed-phase LC was demonstrated to be as a powerful tool for monitoring rapid oxidation reactions of tocopherols with active nitrogen species.

© 2011 Elsevier B.V. All rights reserved.

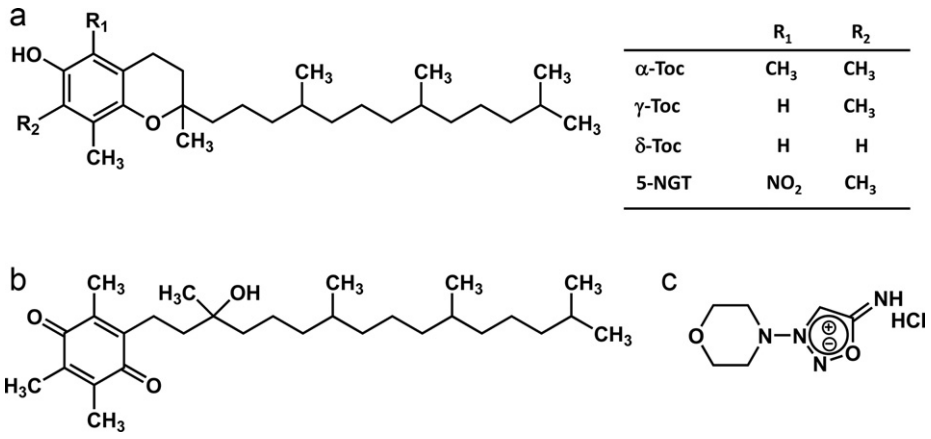
1. Introduction

Nitric oxide (NO) is one of the biological radicals, and has many physiological roles in signal transduction, vascular function, and neurological function [1–3]. It can be readily converted to a variety of reactive nitrogen species, such as dinitrogen trioxide (N_2O_3), nitrogen dioxide (NO_2) and the peroxynitrite anion ($ONOO^-$) by a reaction with oxygen and/or other active oxygen species. These reactive nitrogen species have been shown to cause a serious damage to the body, such as inflammation, diabetes, and neurodegenerative disorders (ALS or Alzheimer's disease) [4–6]. To defend the living system from reactive nitrogen species, some kinds of vitamins such as vitamin C and vitamin E, and one of co-enzymes, ubiquinol, take an important role as antioxidant compounds. Among these essential materials, vitamin E (tocopherol) is a representative antioxidant that works as an effective chain-breaking agent in cell membranes [7]. It contributes to membrane stability and defends the living body against various diseases and aging caused by oxidative stress induced by reactive nitrogen and reactive oxygen species [8]. There are eight isomers of tocopherols. They are α-, β-, γ- and δ-tocopherol, and α-, β-, γ- and δ-tocotrienol, each of which has different biological activities. As a radical scavenger, γ-tocopherol (γ-Toc) is the most effective in these vitamin E isomers [9,10]. Furthermore, Saldeen and coworkers investigated the effects of α-tocopherol (α-Toc) alone and a mixed tocopherol (α-, γ-, and δ-Toc) on hydrogen peroxide-induced lipid peroxidation in human erythrocytes [11]. As a result, it was shown that the coexistence of α-, γ-, and δ-Toc increased the antioxidant activity compared to that of α-Toc alone. Recently, due to the popularity of health-consciousness and anti-aging, vitamin E-related products, such as supplements have been consumed in daily life. However, the efficacy of some products as antioxidants is unknown [12], and it is required to analyze the bioactivities of these antioxidants and their reaction products produced via their oxidation. Monitoring the whole reaction process of tocopherol oxidation has seldom been studied, although some qualitative analyses have been reported [13]. Because tocopherol and its oxidative products have various polarities, it takes a long analysis time for their simultaneous analysis by conventional HPLC (up to 40 min in the case of succinyl tocopheroate [14] and 50 min for tocopherol [13]). Based on this background, we have studied an efficient analytical method for monitoring the time-dependent changes of tocopherol and its oxidized product concentrations using ultra high-speed HPLC analysis.

An ultra-high speed liquid chromatography has become increasingly popular in the research field of HPLC [15–18]. An ultra high-speed chromatographic system enables us to perform fast and efficient separation over an expansive range of flow rate and pressure. This performance was achieved by using a smaller particle size of column packing, which has been applied to the high-throughput screening other than ultra high-speed LC. In general, conventional HPLC separation has been performed on columns packed with 5 μm particles. However, conventional HPLC requires a long analysis time and a large amount of solvents. In the case of ultra high-

* Corresponding author. Tel.: +81 3 5400 2657; fax: +81 3 5400 1378.
E-mail address: kanazawa-hd@pha.keio.ac.jp (H. Kanazawa).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.12.036



	R_1	R_2
α-Toc	CH_3	CH_3
γ-Toc	H	CH_3
δ-Toc	H	H
5-NGT	NO_2	CH_3

Fig. 1. Chemical structures of various types of tocopherols (a), α-tocopheryl quinone (α-TQ) (b) and 3-(4-morpholinyl)sydnonimine hydrochloride (SIN-1) (c).

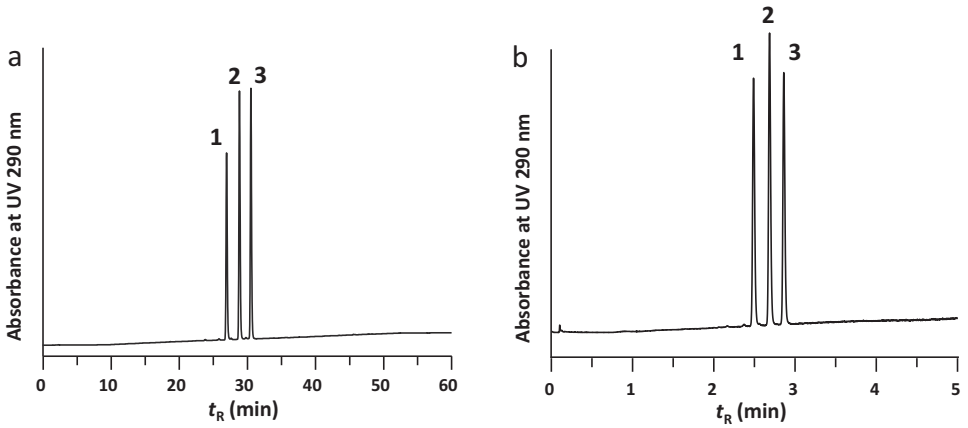


Fig. 2. Typical chromatograms of δ-Toc (1), γ-Toc (2) and α-Toc (3) obtained by the conventional HPLC system (a) and by an ultra high-speed HPLC system (b) with gradient elution. HPLC condition (a): column: LaChrom Ultra C18 (4.6 mm i.d. × 150 mm, 5 μm), flow rate: 1.0 mL/min, column temperature: 30 °C, UV detection: 290 nm, mobile phase: (A) MeCN–H_2O (4:1, v/v), (B) MeCN–diethyl ether (2:3, v/v), gradient condition: 0% (B) up to 100% (B) over the 50 min and 100% (B) isocratic over 50–55 min. HPLC condition (b): column: LaChrom Ultra C18 (2.0 mm i.d. × 50 mm, 2 μm), flow rate: 1.0 mL/min, column temperature: 30 °C, UV detection: 290 nm, mobile phase: (A) MeCN–H_2O (4:1, v/v), (B) MeCN–diethyl ether (2:3, v/v), gradient condition: 0% (B) up to 100% (B) over the 5 min. Concentration of tocopherols: 0.5 mM α-Toc, 0.5 mM γ-Toc, 0.5 mM δ-Toc.

speed LC systems, a column containing particles whose diameters are equal to or less than 2 μm afford excellent chromatographic efficiency at a higher flow rate. As a result, an increased analysis speed and higher resolution are both possible by using the present system.

We have been investigated anti-oxidative effects of tocopherol against reactive nitrogen species [19]. In this article, we studied the high-speed analysis of the products obtained from reaction of α-, γ- and δ-tocopherol with peroxynitrite. More than a 10-fold reduction in the analysis time was observed with good sensitivity in the analysis. Furthermore, we described the application of the present chromatographic system to rapid monitoring for the reaction process involving of the oxidation of tocopherol.

2. Experimental

2.1. Materials and chemicals

α-, γ- and δ-Tocopherol (α-, γ- and δ-Toc) were purchased from Sigma–Aldrich Inc. α-Tocopheryl quinone (α-TQ) was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Table 1
Within- and between-day precision and accuracy of the quantitative analysis of tocopherols.

Actual	Observed	α-Toc RSD (%)	Bias (%)	Observed	γ-Toc RSD (%)	Bias (%)	Observed	δ-Toc RSD (%)	Bias (%)
Concentration (mmol/L)									
Within-day (n = 6)									
0.025	0.02762	0.74	9.5	0.02791	0.7	10.41	0.02782	1.00	10.13
0.05	0.05013	0.51	0.27	0.05134	0.39	2.61	0.05185	0.58	3.57
0.1	0.10210	0.22	2.06	0.10073	0.22	0.72	0.10197	0.29	1.93
0.2	0.19638	0.14	−1.84	0.19608	0.13	−2.00	0.19635	0.16	−1.86
0.4	0.39562	0.08	−1.11	0.39661	0.08	−0.86	0.39664	0.09	−0.85
0.8	0.80274	0.05	0.34	0.80241	0.05	0.27	0.80214	0.05	0.27
Intra-day (n = 6)									
0.025	0.02815	0.74	9.5	0.02799	1.65	10.7	0.02781	1.29	10.1
0.2	0.19674	0.27	−1.66	0.19672	0.18	−1.67	0.19758	0.77	−1.22
0.8	0.80232	0.05	0.29	0.80013	0.15	0.02	0.79959	0.25	−0.05

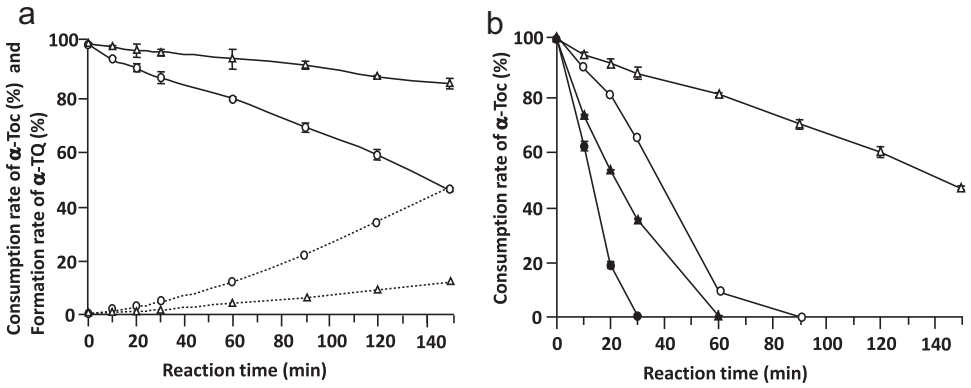


Fig. 3. Monitoring of the reaction of α-Toc with SIN-1. 1 mM (open triangle) and 10 mM (open circle) SIN-1 aqueous solution were used for choosing the optimal SIN-1 concentration. The solid and dotted lines represent the consumption of α-Toc and the formation of α-TQ, respectively (a). Oxidation reactions were carried out at four different temperatures (25 °C: open triangle, 30 °C: open circle, 37 °C: closed triangle, 40 °C: closed circle) (b).

3-(4-Morpholinyl)sydnonimine hydrochloride (SIN-1) was purchased from DOJINDO (Kumamoto, Japan). 5-Nitro-γ-tocopherol (5-NGT) was isolated from the mixture, and purified by preparative TLC and preparative HPLC before being used as the standard sample. Acetonitrile (MeCN) and methanol (MeOH) were of HPLC grade, and diethyl ether was the first grade obtained from Wako Pure Chemical Industries. All other reagents and solvents were of analytical grade.

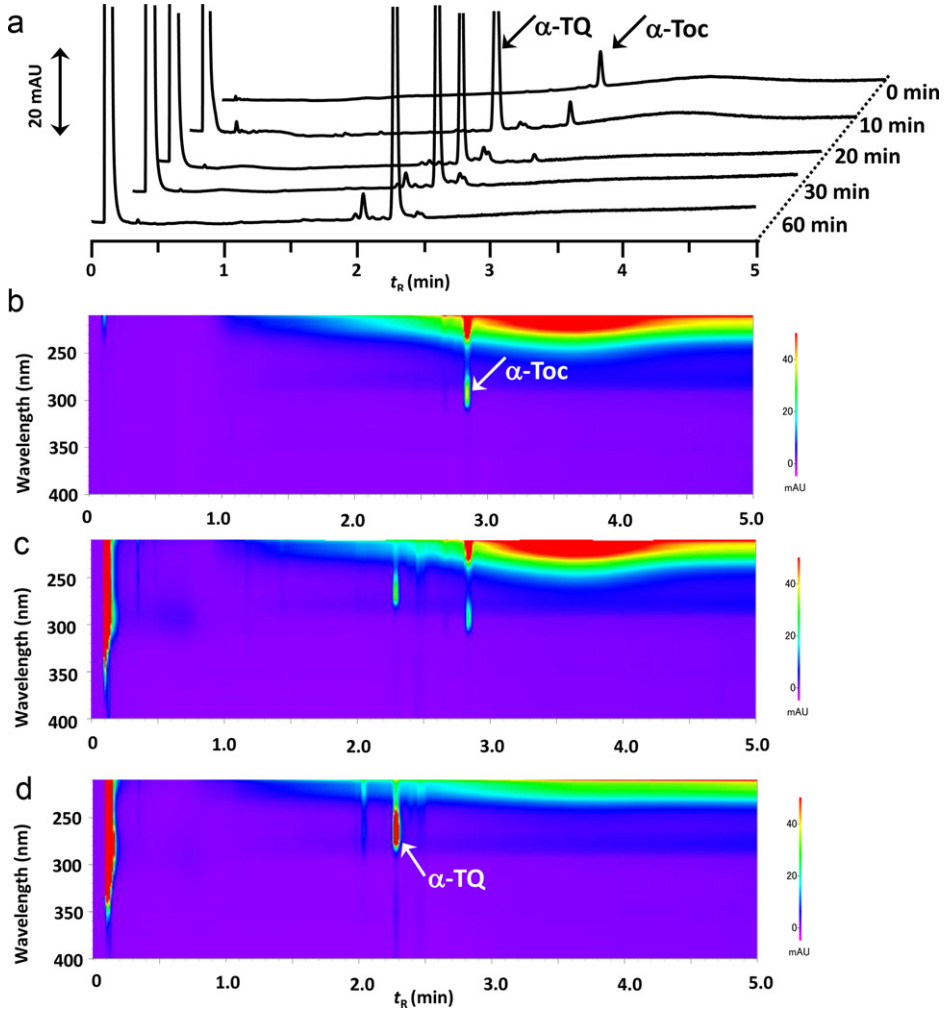


Fig. 4. Rapid monitoring of the reaction of α-Toc with SIN-1 and the obtained chromatogram of UV detection at 268 nm (a) and PDA multichannel chromatograms from 200 to 400 nm [(b) 0 min, (c) 10 min and (d) 60 min]. HPLC condition: column: LaChrom Ultra C18 (2.0 mm i.d. × 50 mm, 2 μm), flow rate: 1.0 mL/min, column temperature: 30 °C, mobile phase: (A) MeCN–H_2O (4:1, v/v), (B) MeCN–diethyl ether (2:3, v/v), gradient condition: 0% (B) up to 100% (B) over the 5 min.

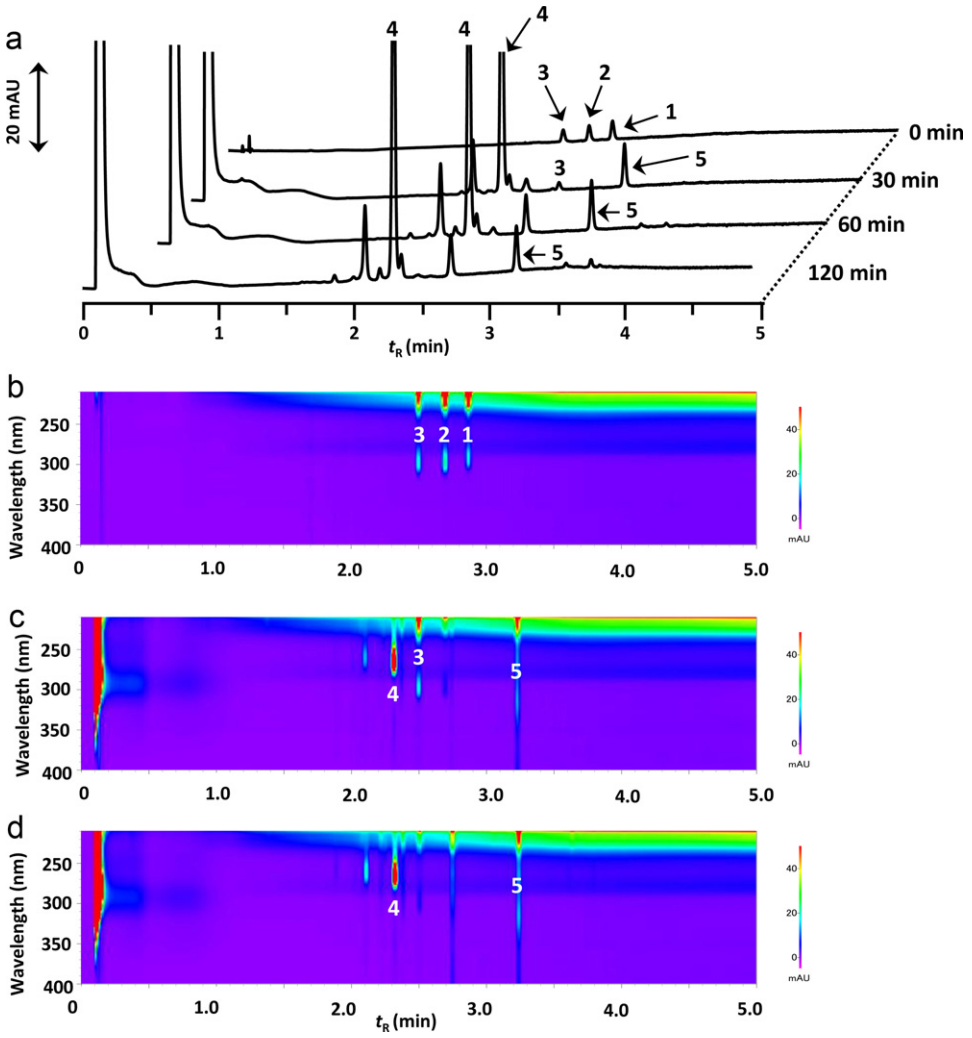


Fig. 5. Rapid monitoring of the reaction of coexistence mixtures of α-, γ- and δ-Toc with SIN-1 and obtained chromatogram of UV detection at 268 nm (a) and PDA multichannel chromatograms from 200 to 400 nm [(b) 0 min, (c) 10 min and (d) 60 min]. HPLC condition: column: LaChrom Ultra C18 (2.0 mm i.d. × 50 mm, 2 μm), flow rate: 1.0 mL/min, column temperature: 30 °C, mobile phase: (A) MeCN–H_2O (4:1, v/v), (B) MeCN–diethyl ether (2:3, v/v), gradient condition: 0% (B) up to 100% (B) over the 5 min. 1: α-Toc, 2: γ-Toc, 3: δ-Toc, 4: α-TQ, 5: 5-NGT.

2.2. Standard solution

Standard solution was prepared by dissolving 5 mmol of α-Toc, 5 mmol of γ-Toc, and 5 mmol of δ-Toc to 5 mL of CH_3CN (α, γ, δ-tocopherol mixed solution). The mixed solution was diluted to the various standard solutions (0.8, 0.4, 0.2, 0.1, 0.05, 0.025 mM).

2.3. Ultra high-speed HPLC condition

The ultra high-speed LC system utilized in this research was a LaChrom Ultra system (Hitachi, Tokyo, Japan) consisting of an L-2160 pump, capable of pumping 60 MPa, an L-2300 column oven, an L-2400U UV detector, an L-2455U diode array detector, an L-2200U auto sampler, and the EZChrom Elite™ for a Hitachi chromatography data system. A LaChrom Ultra C18 (2.0 mm i.d. × 50 mm, 2 μm)

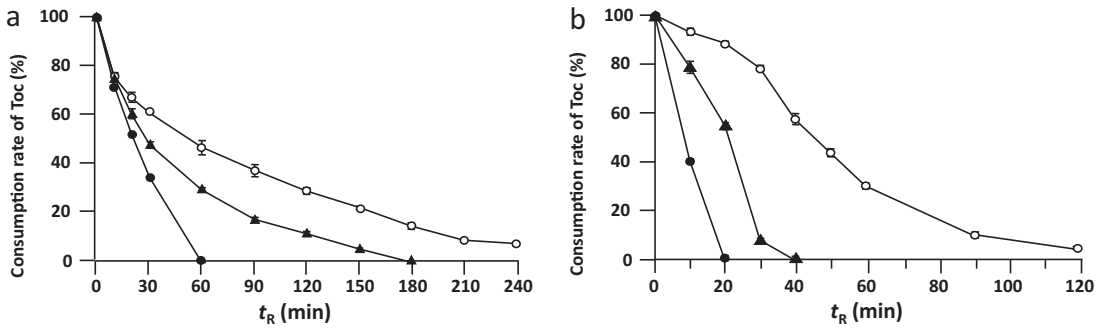


Fig. 6. Time-depended existence rate of tocopherol in the reaction with SIN-1. Individual tocopherols (0.5 mM each concentration) (a), and coexistence mixtures of α-, γ- and δ-Toc (0.5 mM total concentration) (b) were reacted with SIN-1. α-Toc: closed circle, γ-Toc: closed triangle, δ-Toc: open circle.

was used at a flow rate of 1.0 mL/min. The column oven was set at 30 °C and a mobile phase was used as a gradient mode, as follows: mobile phase (A) was MeCN–H_2O (4:1, v/v), mobile phase (B) was MeCN–diethyl ether (2:3, v/v). The gradient ran from 0% (B) up to 100% (B) over a 5 min period. Thereafter, the gradient was returned to the mobile phase (A) to prepare for the next run. The injection volume was 2 μL. The reaction progress was monitored at 290 nm and 268 nm.

2.4. Conventional HPLC condition

The conventional HPLC system utilized in this research was a Hitachi LC system, which was composed of an L-6300 intelligent pump, an L-7400 UV detector and a D-2000 Elite™ for the data system. A SSC-2300 (Senshu Scientific Co. Ltd., Tokyo, Japan) column oven was used at 30 °C. A LaChrom Ultra C18 (4.6 mm i.d. × 150 mm, 5 μm) was used at a flow rate of 1.0 mL/min. The mobile phase was used as a gradient mode as follows: mobile phase (A) was MeCN–H_2O (4:1), mobile phase (B) was MeCN–diethyl ether (2:3). The gradient ran from 0% (B) up to 100% (B) over a period of 50 min and 100% (B) isocratic over 50–55 min. Thereafter, the gradient was returned to the mobile phase (A) to prepare for the next run. The injection volume was 10 μL.

2.5. General procedure of the reaction of tocopherols with 3-morpholinosydnomine (SIN-1)

The reaction was started with the addition of an SIN-1 aqueous solution to the tocopherol solution [MeCN–$CH_3CO_2NH_4$ (pH 7.4, 20 mM) (3:1, v/v)] in a screw vial equipped with a septum and cap. Material sampling was carried out using a Hamilton gas-tight syringe. The sample solutions were filtered through the Ekicrodisc® 13CR (0.2 μm, PTFE membrane, Japan Pall, Ibaragi, Japan) and injected to an ultra high-speed HPLC system.

2.6. Optimization of the reaction condition

The reaction of SIN-1 and tocopherols was optimized while varying the SIN-1 concentration and the reaction temperature. Firstly, a 1 mM or 10 mM SIN-1 aqueous solution was added to the 0.5 mM α-Toc [MeCN–$CH_3CO_2NH_4$ (pH 7.4, 20 mM) (3:1, v/v)] solution at room temperature. Material sampling was carried out after starting the reaction from 0 to 120 min (0, 10, 20, 30, 60, 90 and 120 min). Secondly, a 10 mM SIN-1 aqueous solution was added to the 0.5 mM α-Toc solution and reacted at various temperatures (25, 30, 37 and 40 °C). Material sampling was carried out after starting the reaction from 0 to 120 min (0, 10, 20, 30, 60, 90 and 120 min).

2.7. Reaction of SIN-1 with tocopherol

The reaction was started with the addition of a 10 mM SIN-1 aqueous solution to the 0.5 mM α-, γ- or δ-Toc [MeCN–$CH_3CO_2NH_4$ (pH 7.4, 20 mM) (3:1, v/v)] solution at 37 °C. Material sampling was carried out after starting the reaction from 0 to 240 min (0, 10, 20, 30, 60, 90, 120, 150, 180, 210 and 240 min).

The reaction of SIN-1 and a mixture of α-, γ- and δ-Toc was conducted as described above, except for the concentration of each Toc was set as a 0.17 mM solution [MeCN–$CH_3CO_2NH_4$ (pH 7.4, 20 mM) (3:1, v/v)]. Material sampling was carried out after starting the reaction from 0 to 120 min (0, 10, 20, 30, 40, 60, 90 and 120 min).

3. Results and discussion

3.1. Ultra high-speed analysis of tocopherols

Structures of tocopherols used in this research are shown in Fig. 1. In order to monitor the reaction process of tocopherols and reactive nitrogen species, short-time analysis was required because this oxidation reaction occurs promptly and produces many kinds of oxidants. Fig. 2 shows chromatograms of α-, γ- and δ-Toc obtained from the conventional HPLC method and the ultra high-speed LC methods on smaller columns packed with 2 μm particles. Under these analytical conditions, the flow rate and mobile-phase composition were entirely the same, and only the particle size of the column packing was different. The most obvious advantage of using high-speed LC methods was a reduction in the separation time without reducing the separation efficiency. Concerning the conventional method, it took more than 30 min to conduct one analysis (δ-Toc: 28.2 min, γ-Toc: 30.3 min, α-Toc: 32.3 min). On the contrary, it took only a 5 min analysis time for high-speed LC methods (δ-Toc: 2.5 min, γ-Toc: 2.7 min, α-Toc: 2.9 min) and accomplished reduction of the analysis time was to one-tenth. Ultra high-speed HPLC had a possibility to monitor short-lived reaction intermediates of tocopherols.

3.2. Calibration curves and precision

The peak areas were calculated on the chromatogram of each product or the starting material at 268 nm or 290 nm. The linear relationship between the amounts of the compound and the peak areas in the UV chromatogram were obtained between 0.025 mmol/L and 0.8 mmol/L (α-Toc, γ-Toc, δ-Toc). In the case of α-TQ, one-point determination was carried out using 0.5 mmol/L concentration. These results were reproducible in all cases. The linear relationship calculated between the peak area (y) and the concentration (x mol/L) of each product (α-Toc, γ-Toc, δ-Toc, α-TQ) were as follows:

α-Toc (λ = 290 nm); $y = 899{,}617x - 3.6363 \times 10^3$ $R^2 = 0.9999$.
γ-Toc (λ = 290 nm); $y = 946{,}885x + 6.1232 \times 10^2$ $R^2 = 0.9999$.
δ-Toc (λ = 290 nm); $y = 794{,}326x + 1.410 \times 10^3$ $R^2 = 0.9999$.
α-TQ (λ = 268 nm); $y = 3{,}325{,}997x$ $R^2 = 1.0000$.

The within- and between-day precision (RSD) calculated during replicate assays ($n = 6$) of tocopherols was less than 1.7% over a wide range of concentrations (Table 1). The within- and between-day bias assessed during the replicate assays for various tocopherols varied between 0 and 3.6% except the data obtained at 0.025 mmol/L. The data indicated that the developed HPLC method is reproducible and accurate.

3.3. Optimization of the oxidation reaction using SIN-1

Before monitoring the reaction with tocopherol and peroxynitrite, we studied the reaction conditions by varying the oxidant concentration and reaction temperature.

We chose SIN-1 as a reactive nitrogen species-generating agent (Fig. 1(c)). At physical or alkaline pH, SIN-1 undergoes rapid hydrolysis to the ring-opened form from which the NO radical is released. The activation of SIN-1 also produces the superoxide anion, which combines with NO to produce the highly reactive peroxynitrite anion [20]. Using a constant amount of α-Toc (0.5 mM), different concentrations of SIN-1 aqueous solutions (1 mM or 10 mM) were added, and temporal sampling from the reaction solution was carried out. Fig. 3(a) shows the time-dependent decrease of the α-Toc and increase of α-tocopheryl quinone (α-TQ), which was a major oxidation product, monitored by the ultra high-speed HPLC

method. On 1 mM SIN-1 concentration, the temporal consumption rate of α-Toc was moderate and α-Toc remained approximately 75% after 120 min. Under a high concentration of the SIN-1 condition, α-Toc rapidly decreased and remained 20% within 120 min. It was obvious that the reaction rate of α-Toc increased with a higher concentration of SIN-1. Thus, the use of a 10 mM concentration of SIN-1 for the following reaction was decided. Next, to optimize the reaction temperature, the reaction of α-Toc with SIN-1 was conducted at different temperatures (25, 30, 37, 40 °C) (Fig. 3(b)). These results showed that the rising of the reaction temperature accelerated the oxidation rate of α-Toc. Considering both the measurement intervals and the monitoring time, the reaction condition was decided as the SIN-1 concentration of 10 mM and reaction temperature of 37 °C, respectively.

3.4. Monitoring of the reaction with tocopherol and SIN-1

As described above, the ultra high-speed HPLC conditions and optimal reaction conditions were decided, thus we conducted HPLC monitoring of the reaction with tocopherols and SIN-1. Firstly, we carried out the reaction of individual tocopherol with SIN-1 and monitored the consumption rate of each tocopherol. Fig. 4(a) shows a typical chromatogram obtained from the reaction with α-Toc and SIN-1, at UV 268 nm detection. α-Toc was consumed rapidly after starting, and its peak became unobservable within 30 min. In addition, a new product peak mainly appeared at a retention time (t_R) of 2.3 min. This product was identified as α-tocopheryl quinone (α-TQ) by a agreement of the absorption spectrum and t_R on HPLC analysis of an α-TQ standard sample. α-TQ is well known as the reaction product of oxidized α-Toc [21], and is considered to be as an anti-oxidant agent, which collaborates with α-Toc. [22]. The wavelengths of the maximum absorption of tocopherols and its derivative are often different, multiple wavelength detection is considered to be useful for analyzing the qualitative analysis of complicated oxidation reactions. For this reason, we applied a photo-diode array (PDA) detector to monitor the reaction process (Fig. 4(b)–(d)). The PDA detector covered the wavelength range from 200 to 400 nm, and the signals of α-Toc and α-TQ were easily detected in multichannel chromatograms. The reaction monitoring of γ- or δ-Toc analysis was carried out, and their time-consumption curve is summarized in Fig. 6(a). In these results, the consumption rate of α-Toc was fastest among all tocopherols. All of the reactions were completed within 240 min.

Next, SIN-1 oxidation reaction was carried out using coexistence mixtures of α-, γ- and δ-Toc solutions (total tocopherol concentration were of 0.5 mM). Fig. 5 shows the monitoring result of three tocopherols and their reaction products. Many reaction products were produced immediately after starting the reaction. Concerning the parent tocopherols, though α- and γ-Toc were almost consumed, δ-Toc remained at least 5% after 120 min. Concurrently, α-TQ (t_R: 2.3 min) and 5-nitro-γ-tocopherol (5-NGT, t_R: 3.3 min), which were derived from α- and γ-Toc, respectively, were obviously detected. The time-dependent existence rate of each tocopherol is summarized in Fig. 6(b). Compared with Fig. 6(a), though the order of the consumption rate of tocopherols was the same (α-Toc > γ-Toc > δ-Toc), δ-Toc each remained after 120 min, despite that its concentration was one-third of that used in each individual reaction. Because mixed tocopherols have a stronger inhibitory effect on peroxidation than one tocopherol alone [11], the obtained result suggested that long-life δ-Toc played a key role in to such a phenomenon. A more detailed study of the reaction mechanism of tocopherol and reactive nitrogen species is now in progress in our laboratory.

In conclusion, we applied the ultra high-speed chromatographic system to monitoring the reaction of tocopherol and SIN-1. Using the ultra high-speed LC system equipped with a PDA detector, a low dead-volume system configuration and the detection of a wide range of analyte was accomplished. Moreover, one cycle analysis time of tocopherol and its major oxidation product was greatly shortened (by more than 10 times). We could trace the decreasing of tocopherols and the generation of their oxidant product efficiently, and could observe the consumption rate of α-, γ- and δ-tocopherols. The ultra high-speed LC was found to be a powerful tool for monitoring of rapid oxidation reaction of tocopherols and its related antioxidant compounds. The applications of the present system to more complicated reaction monitoring will appear in due course.

Acknowledgement

Technical assistance of Ms. Rie Tanahashi is gratefully acknowledged.

References

[1] L.J. Ignarro, Nitric oxide a unique endogenous signaling molecule in vascular biology, Angew. Chem. Int. Ed. Engl. 38 (1999) 1882–1892.
[2] L.A. Ridnour, J.S. Isenberg, M.G. Espey, D.D. Thomas, D.D. Roberts, D.A. Wink, Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 13147–13152.
[3] K. Yamashiro, A.B. Wilson, J. Duchene, C. Panayiotou, T. Urabe, N. Hattori, A. Ahlamalia, Alternation in nitric oxide and endothelin-1 bioactivity underlie cerebrovascular dysfunction in ApoE-deficient time, J. Cereb. Blood Flow Metab. 30 (2010) 4639–4656.
[4] H. Wiseman, B. Halliwell, Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer, Biochem. J. 313 (1996) 17–29.
[5] W. Kossenjans, A. Eis, R. Sahay, D. Brochman, L. Myatt, Role of peroxynitrite in altered fetal-placental vascular reactivity in diabetes or preeclampsia, Am. J. Physiol.: Heart Circ. Physiol. 278 (2000) 1311–1319.
[6] J.S. Beckman, M. Carson, C.D. Smith, W.H. Koppenol, ALS, SOD and peroxynitrite, Nature 364 (1993) 584.
[7] M. Meydani, E. Vitamin, Lancet 345 (1995) 170–175.
[8] K. Saldeen, T. Saldeen, Importance of tocopherols beyond α-tocopherol: evidence from animal and human studies, Nutr. Res. 25 (2005) 877–889.
[9] G.G. Duthie, B.M. Gonzalez, P.C. Morrice, Inhibitory effects of isomers of tocopherol on lipid peroxidation of microsomes from vitamin E-deficient rat, Free Radic. Res. Commun. 15 (1991) 35–40.
[10] G. Wolf, Gamma-tocopherol: an efficient protector to lipid against nitric oxide-mediated peroxidative damage, Nutr. Rev. 55 (1997) 376–378.
[11] M. Liu, R. Wallin, A. Wallmon, T. Saldeen, Mixed tocopherols have a stronger inhibitory effect on lipid peroxidation than α-tocopherol alone, J. Cardiovasc. Pharmacol. 39 (2002) 714–721.
[12] M.C. Morris, D.A. Evans, C.C. Tangney, J.L. Bienias, R.S. Wilson, N.T. Aggarwal, P.A. Scherr, Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change, Am. J. Clin. Nutr. 81 (2005) 508–514.
[13] Y. Nagata, Y. Matsumoto, H. Kanazawa, Analysis of the products of reaction between α- or γ-tocopherol and nitrogen oxides using HPLC with UV–VIS and APCI-MS detection, J. Chromatogr. A 1036 (2004) 177–182.
[14] R.L. Good, K.A. Roupe, C. Fukuda, G.D. Clifton, M.W. Fariss, N.M. Davies, Direct high-performance liquid chromatographic analysis of D-tocopheryl acid succinate and derivatives, J. Pharm. Biomed. Anal. 39 (2005) 33–38.
[15] J.E. McNair, K.C. Lewis, J.W. Joregenson, Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns, Anal. Chem. 69 (1997) 983–989.
[16] T. Yoshida, R.E. Majors, High-speed analyses using rapid resolution liquid chromatography on 1.8 micron porous particles, J. Sep. Sci. 29 (2006) 2421–2432.
[17] A. de Villiers, F. Lestremaub, R. Szucs, S. Gélébartb, F. Davida, P. Sandra, Evaluation of ultra performance liquid chromatography. Part I. Possibilities and limitations, J. Chromatogr. A. 1127 (2006) 60–69.
[18] M. Ito, K. Toyosaki, K. Shimizu, Ultra high-speed chromatographic system, LaChromUltra, Chromatography 28 (2007) 101–104.
[19] Y. Nagata, T. Nishio, S. Matsumoto, H. Kanazawa, M. Mochizuki, Y. Matsushima, Reaction of 2,2,5,7,8-pentamethyl-6-chromanol, an α-tocopherol analogue, with NO in the presence of oxygen, Bioorg. Med. Chem. Lett. 10 (2000) 2709–2712.
[20] K. Schonafinger, Heterocyclic NO prodrugs, IL Farmaco 54 (1999) 316–320.
[21] H. Kanazawa, C. Miyata, Y. Nagata, S. Urano, Y. Matsushima, Determination of α-tocopherol and α-tocopherylquinone in rat tissues and plasma by high-performance liquid chromatography with electrochemical detection, Chem. Pharm. Bull. 48 (2000) 1462–1466.
[22] L. Gille, K. Staniek, T. Rosenau, J.C. Duvigneau, A.V. Kozlov, Tocopheryl quinones and mitochondria, Mol. Nutr. Food Res. 54 (2010) 601–615.