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Analyses for phosphatidylcholine hydroperoxides by LC/MS☆

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ABSTRACT

A new liquid chromatography mass spectrometry (LC/MS) method has been developed for the qualitative and quantitative analyses of phosphatidylcholine hydroperoxides (PC-OOH) in human plasma using a synthetic hydroperoxide (1-stearoyl-2-erucoyl-PC monohydroperoxide, PC 18:0/22:1-OOH) as an internal standard. 1-Stearoyl-2-linoleoyl-PC monohydroperoxide (PC 18:0/18:2-OOH) was identified in plasma by LC/MS by comparison with an authentic standard. The calibration curves obtained for 1-palmitoyl-2-linoleoyl-PC monohydroperoxide, PC 16:0/18:2-OOH and PC 18:0/18:2-OOH were linear throughout the calibration range (0.1–1.0 pmol). The limit of detection (LOD) (S/N = 3:1) was 0.01 pmol, and the limit of quantification (LOQ) (S/N = 6:1) was 0.1 pmol for both PC 16:0/18:2-OOH and PC 18:0/18:2-OOH. Plasma concentrations of PC 16:0/18:2-OOH and PC 18:0/18:2-OOH were 89 and 32 nM, respectively, in a healthy volunteer.

1. Introduction

Lipid peroxidation has been suggested to play a significant role in the pathophysiology of atherosclerosis, diabetes, aging and other conditions [1]. In recent years, Alzheimer's disease has been associated with increased lipid peroxidation and decreased polyunsaturated fatty acids [2,3]. Several studies have reported the formation of hydroperoxides of cholesteryl ester, triglyceride and phosphatidylcholine (PC) as primary lipid oxidation products [4–7]. In particular, PC hydroperoxides (PC-OOH) and other oxidized PC have been the foci of lipid peroxidation studies because their precursor, PC, is a major component of cellular membranes and an important constituent of serum lipoproteins *in vivo*. High performance liquid chromatography with chemiluminescence detection has been reported for quantitative analyses of PC-OOH [8], but the technique is unable to provide structurally characteristic data for individual PC-OOH components. In recent years, mass spectrometry (MS) has become a powerful tool for structural identification of phospholipids. Several groups have completed detailed structural determination of oxidized PC and phosphatidylethanolamine using liquid chromatography mass spectrometry (LC/MS) in positive and negative modes [9–14]. To the best of our knowledge, there has not yet been a quantitative LC/MS method reported for PC-OOH using an authentic PC-OOH as the internal standard.

Suitably sensitive quantitative methods of analysis for PC-OOH are required to allow the analysis of physiological samples for the presence of PC-OOH and to allow the measurement of the oxidative stress signals for disease state monitoring and enable new understandings of the pathogenesis process. It is well recognized that lipid hydroperoxides are chemically unstable and consequently may be lost during analysis. The problem may be overcome by the use of a non-endogenous PC-OOH as an internal standard, which can correct for extraction efficiency, chemical stability and ionization efficiency.

Here we report both qualitative and quantitative analyses of PC-OOH in human plasma by LC/MS system with a synthetic internal standard.

2. Experimental

2.1. Materials

1-Palmitoyl-2-linoleoyl-PC monohydroperoxide (PC 16:0/18:2-OOH), 1-stearoyl-2-linoleoyl-PC monohydroperoxide (PC

Abbreviations: PC, phosphatidylcholine(s); PC-OOH, phosphatidylcholine hydroperoxide(s); PC 16:0/18:2-OOH, 1-palmitoyl-2-linoleoyl-phosphatidylcholine monohydroperoxide; PC 18:0/18:2-OOH, 1-stearoyl-2-linoleoyl-phosphatidylcholine monohydroperoxide; PC 18:0/22:1-OOH, 1-stearoyl-2-erucoyl-phosphatidylcholine monohydroperoxide; HPLC, high performance liquid chromatography; IS, internal standard; ESI-MS, electrospray ionization-mass spectrometry; SRM, selected reaction monitoring; LOD, limit of detection; LOQ, limit of quantification.

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18:0/18:2-OOH) and, 1-stearoyl-2-erucoyl-PC monohydroperoxide (PC 18:0/22:1-OOH, as the internal standard, IS) were synthesized by photosensitized oxidation as described previously [8]. All other chemicals and solvents used were analytical grade and obtained from Wako Pure Chemical Industry, Ltd. (Osaka, Japan).

2.2. Extraction of PC-OOH from human plasma

A healthy volunteer (45 years old, female) underwent an overnight fast and her blood (2 mL) was drawn into tubes containing EDTA-2Na. Plasma was separated by centrifugation (2000 × *g*, 10 min, 4 °C), and immediately stored at −80 °C in two 0.5 mL aliquots. Extraction was started within 2 min after plasma was taken out of an −80 °C freezer. For quantitative analysis, the plasma sample (0.5 mL) was mixed with 500 pmol IS (50 pmol/μL in acetonitrile), 0.5 mL freshly prepared 0.005% 2,6-di-*tert*-butyl-*p*-cresol (antioxidant) in acetonitrile, and 5.0 mL chloroform; for qualitative analysis, the plasma sample (0.5 mL) was mixed with 0.5 mL freshly prepared 0.005% 2,6-di-*tert*-butyl-*p*-cresol in acetonitrile, and 5.0 mL chloroform. The mixture was vigorously mixed for 0.5 min and then centrifuged at 2000 × *g* for 5 min at 4 °C. The chloroform layer was collected and evaporated *in vacuo*. The residue was dissolved in 200 μL of acetonitrile, and a 20 μL portion was injected into the qualitative LC/MS system and a 5 μL portion was injected into the quantitative LC/MS system.

The study protocol was approved by the Ethical Committee, Faculty of Health Sciences, Hokkaido University (approval number 08-57-2). Written informed consent was obtained from the volunteer.

2.3. Qualitative LC/MS system

The qualitative analyses were carried out using a Finnigan LXQ linear ion trap mass spectrometer (Thermo Fisher Scientific Inc. Waltham, MA, USA) equipped with an electrospray ionization-mass spectrometry (ESI-MS) source. The mass range of the instrument was set at *m/z* 180–1000 and scan duration of MS at 0.5 s in positive and negative ion mode. The ionization conditions were as follows: ion source voltage, ±4.5 kV; capillary voltage, ±20 V; capillary temperature, 350 °C; sheath gas (nitrogen) flow rate, 40 arbitrary units (arb. units); auxiliary gas (nitrogen) flow rate, 5 arb. units; tube lens offset voltage, ±70 V. For multistage tandem mass spectrometry analyses, helium gas was used as the collision gas and the pressure was 1.995 mPa. MS/MS experiments were carried out with relative collision energy of 35%.

Reverse-phased LC separation was achieved using a Hypersil Gold column (C8, 100 mm × 2.1 mm I.D., 5 μm, Thermo Fisher Scientific Inc. Waltham, MA, USA) at 60 °C. Gradient elution was performed using a mobile phase comprising 5.0 mM aqueous ammonium acetate (solvent A), acetonitrile (solvent B), and 2-propanol (solvent C). The gradient was programmed as follows: 0.00–6.00 min 20% A and 80% B; 6.01–10.00 min 20% A and 80% C; 10.01–12.00 min 20% A and 80% B. The flow rate was 0.2 mL/min.

2.4. Quantitative LC/MS system

Quantitative analyses were performed using an Accela HPLC system and TSQ Quantum Access triple stage quadrupole mass spectrometer with a heated-ESI (H-ESI) probe (Thermo Fisher Scientific Inc.). The scan duration of MS was set at 0.5 s in positive ion

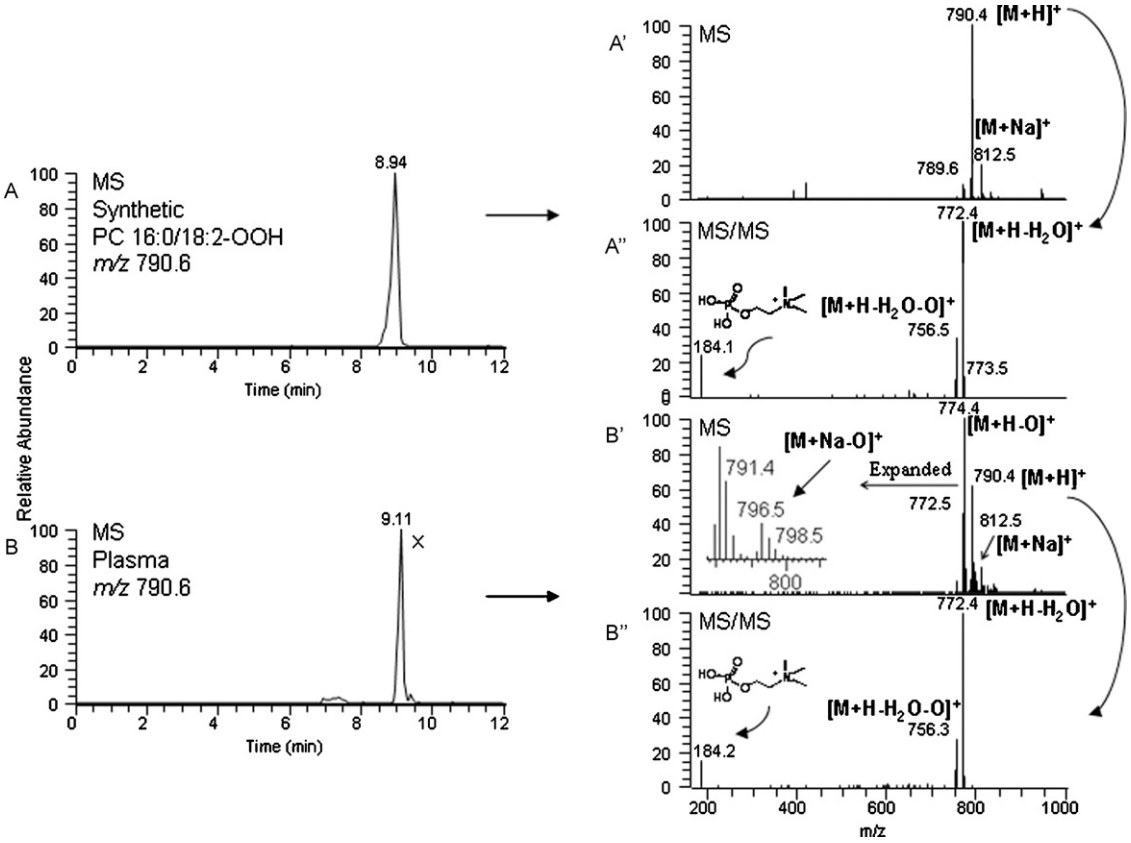

Fig. 1. Qualitative profile obtained from the qualitative LC/MS system in positive mode. (A) and (B): selected ion (*m/z* 790.6) mass chromatograms of synthetic PC 16:0/18:2-OOH (20 pmol) and plasma extract, respectively; (A′) and (B′): selected ion (*m/z* 790.6) MS spectra of the synthetic PC 16:0/18:2-OOH and plasma extract, respectively; (A″) and (B″): selected ion (*m/z* 772.4) MS/MS spectra of synthetic PC 16:0/18:2-OOH and plasma extract, respectively; For abbreviation and LC/MS conditions see text.

Table 1
Diagnostically significant ions of standard and plasma obtained from spectra by qualitative LC–MS system.

	Species	Synthetic PC 16L0/18:2-OOH	Plasma (x)	Synthetic PC 18:0/18:2-OOH	Plasma (y)
MS (*m*/*z*)	$[M+H]^+$	790.4	790.4	818.4	818.4
	$[M+Na]^+$	812.5	812.5	840.5	840.5
	$[M+H-O]^+$	–	774.4	–	802.4
	$[M+Na-O]^+$	–	796.5	–	824.4
MS/MS (*m*/*z*)	Precursor ions	790.4	790.4	818.4	818.4
	$[M+H-H_2O]^+$	772.4	772.4	800.4	800.4
	$[M+H-H_2O-O]^+$	756.5	756.5	784.5	784.5
	$[H_2PO_4(CH_2)_2N(CH_3)_3]^+$	184.1	184.2	184.3	184.3

mode. The ESI inlet conditions were as follows: ion source voltage, ±3.0 kV; vaporizer temperature, 300 °C; sheath gas (nitrogen) pressure, 50 psi; auxiliary gas temperature, 20 psi; capillary temperature, 270 °C; tube lens offset voltage for parent mass at *m*/*z* 790.6, 136 V; *m*/*z* 818.7, 128 V; and *m*/*z* 876.8, 151 V; respectively. Argon was used as the collision gas and the pressure was 1.0 m convert to mPa as above to keep consistent units. The collision energies yielding the product ions *m*/*z* 184.0 from the parent ions *m*/*z* 790.6, *m*/*z* 818.7, and *m*/*z* 876.8 were 136 V, 128 V, and 151 V, respectively.

The LC separations were conducted on a reversed-phase column (Hypersil Gold C8, 50 mm × 2.1 mm I.D., 5 μm,) at 60 °C. The mobile phase consisted of two solvents (A and B); solvent A was 5.0 mM ammonium acetate aqueous solution, solvent B was 2-propanol. The gradient was programmed as follows: 0.00–3.00 min 100% A; 3.01–5.00 min, a linear gradient elution: 5% solution A to 0% A against solution B over 2.99 min; 5.01–8.00 min 100% B, 8.01–10.0 min 100% A. The flow rate was 0.3 mL/min.

3. Results

3.1. Identification of PC 16:0/18:2-OOH in human plasma

Fig. 1A and B shows selected ion chromatograms obtained from a pure standard of PC16:/18:2-OOH and plasma sample, along with their corresponding mass spectra. Peaks in the selected ion chromatograms for *m*/*z* 790.4 were observed at approximately 8.9 and 9.1 min for the standard PC16:0/18:2-OOH (20 pmol) and plasma extract (in Fig. 1A and B). There is a difference of 0.17 min in retention time, indicating that the ion *m*/*z* 790.4 was maybe subject to interference from other endogenous components in the plasma sample. The ESI-mass spectra of synthetic PC 16:0/18:2-OOH and plasma extract show molecular-related ions $[M+H]^+$ and $[M+Na]^+$ at *m*/*z* 790.4 and 812.5, respectively (Fig. 1A′ and B′). An abundant ion at *m*/*z* 774.4 $[M+H]^+$ and a minor ion at *m*/*z* 796.5 $[M+Na]^+$ were observed at the expanded spectrum (Fig. 1B′), reflecting that an alcohol PC 16:0/18:2-OH was derived from PC 16:0/18:2-OOH by loss of oxygen. The MS/MS spectra derived from *m*/*z* 790.4 for

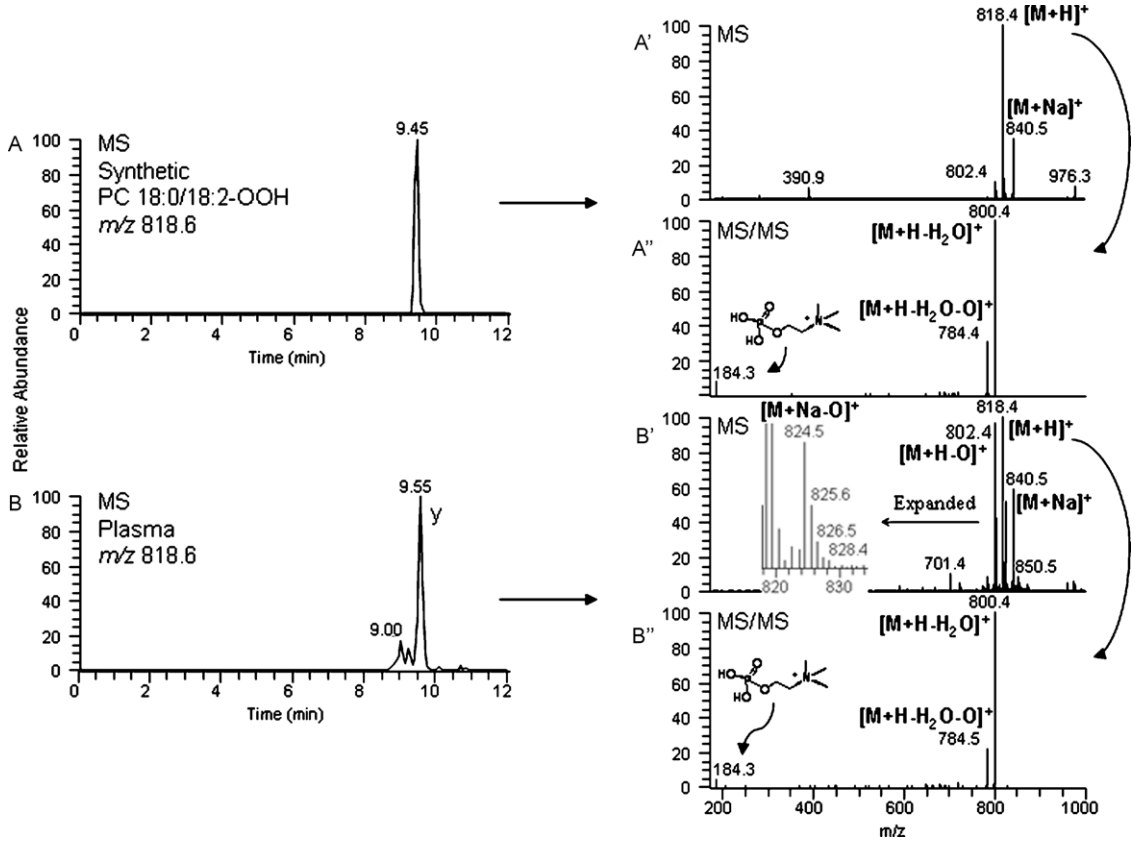

Fig. 2. Qualitative profile obtained from the qualitative LC/MS system in positive mode. (A) and (B): selected ion (*m*/*z* 818.6) mass chromatograms of synthetic PC 18:0/18:2-OOH (20 pmol) and plasma, respectively; (A′) and (B′): selected ion (*m*/*z* 818.6) MS spectra of synthetic PC 18:0/18:2-OOH and plasma, respectively; (A″) and (B″): selected ion (*m*/*z* 800.4) MS/MS spectra of the synthetic PC 18:0/18:2-OOH and plasma, respectively; For abbreviation and LC/MS conditions see text.

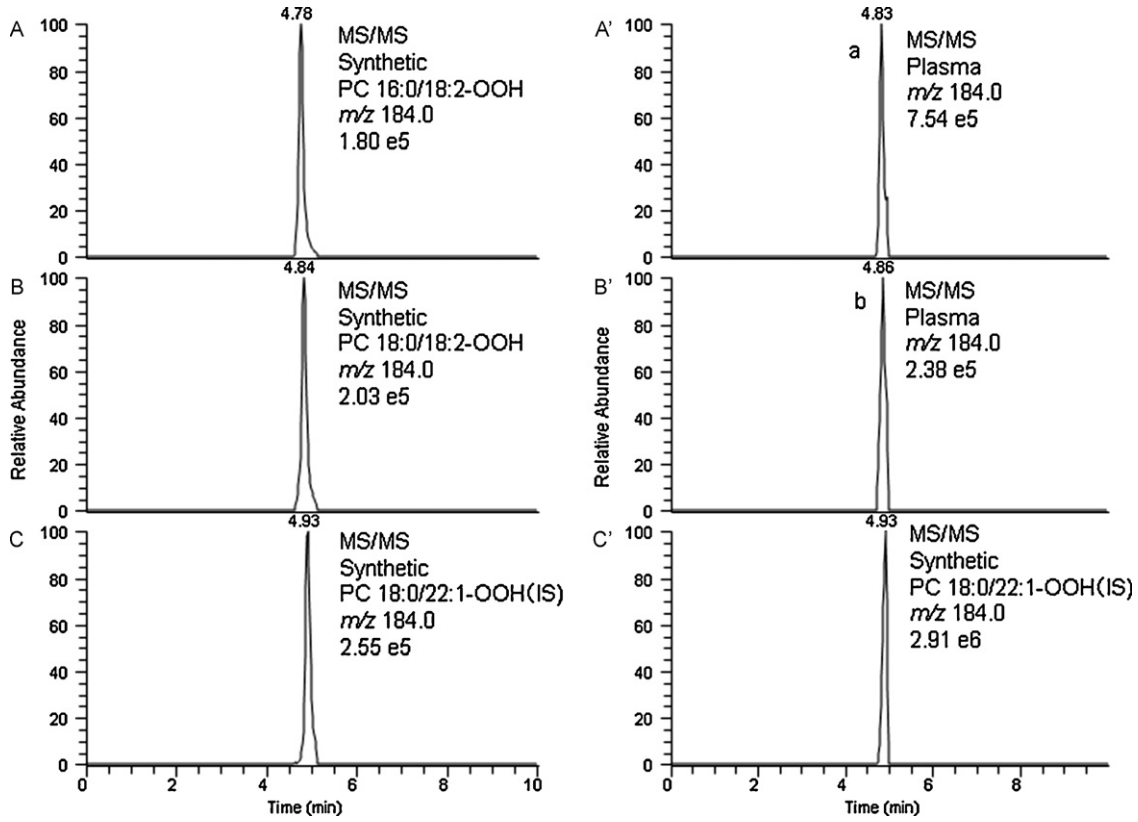

Fig. 3. SRM chromatograms obtained by the quantitative LC/MS system in positive mode. (A): synthetic PC 16:0/18:2-OOH (0.6 pmol); (B): synthetic PC 18:0/18:2-OOH (0.6 pmol); (C): IS (0.6 pmol); (A′): plasma extract (precursor: m/z 790.6); (B′) plasma extract (precursor: m/z 818.6); (C′): plasma extract after addition of the IS (12.5 pmol). For abbreviations and LC/MS conditions see text.

the synthetic PC 16:0/18:2-OOH and plasma extract are shown in Fig. 1A″ and B″, respectively. In Fig. 1A″, the base peak was *m/z* 772.4 corresponding to $[M+H-H_2O]^+$, which derived from *m/z* 790.6, and this breakdown product lost 18 mass units, corresponding to dehydration; the small peak associated with fragment ions appeared at *m/z* 756.5, and this breakdown product more likely that the fragmentation of the peroxy proceeds via loss of H_2O to the intermediate epoxide which could then generate the *m/z* 756.3 by loss of oxygen ($[M+H-H_2O-O]^+$). The fragmentation supported the assignment as hydroperoxide; another small peak was observed at *m/z* 184.1 as choline phosphate. For MS/MS spectrum of plasma extract (Fig. 1B″), the base peak was *m/z* 772.4 as $[M+H-H_2O]^+$; the small peaks *m/z* 756.3 as $[M+H-H_2O-O]^+$ and 184.2. Owing to the diagnostically significant ions of synthetic PC-OOH and plasma from spectra of MS and MS/MS, the peak (x) at 9.11 min in Fig. 1B was identified as the PC 16:0/18:2-OOH. These characteristic ions are summarized in Table 1.

3.2. Identification of PC 18:0/18:2-OOH in human plasma

Fig. 2A and B also shows selected ion chromatograms, and Fig. 2A′, A″, B′, and B″ shows their corresponding spectra obtained from the qualitative LC/MS system in positive ion mode. The peaks in selected ion (*m/z* 818.6) mass chromatograms (Fig. 2A and B) for synthetic PC 18:0/18:2-OOH and plasma extract (peak y) are observed at 9.45 and 9.55 min, respectively. The ESI-mass spectra of synthetic PC 18:0/18:2-OOH and plasma extract also show protonated molecular ion ($[M+H]^+$) and sodiated molecular ion ($[M+Na]^+$) at *m/z* 818.4 and 840.5, respectively, as shown in Fig. 2A′ and B′. A major ion at *m/z* 802.4 $[M+H]^+$ (Fig. 2B′) and a minor ion at 824.4 $[M+Na]^+$ were observed at the expanded spectrum (Fig. 2B′), reflecting a molecular species alcohol PC 18:0/18:2-OH was derived from PC 18:0/18:2-OOH by loss of oxygen. The MS/MS spectra of selected ion (*m/z* 800.4) derived from the above precursor ion (*m/z* 818.6) are shown in Fig. 2A″ (synthetic PC 18:0/18:2-OOH) and Fig. 2B″ (plasma). In spectrum Fig. 2A″, the base peak ion was observed at *m/z* 800.4; the small peak ion associated with base peak appeared at *m/z* 784.4, and this breakdown product more likely that the fragmentation of the peroxy proceeds via loss of H_2O to the intermediate epoxide which could then generate the *m/z* 784.4 by loss of oxygen ($[M+H-H_2O-O]^+$). Another small peak was *m/z* 184.3 as choline phosphate. For MS/MS spectrum of plasma extract, the base peak ion (*m/z* 800.4) and the other fragment ions at *m/z* 784.5 and at *m/z* 184.3 are observed similar to the above standard fragmentation. Thus, the peak (y) in Fig. 2B was identified as the PC 18:0/18:2-OOH. These characteristic ions are also summarized in Table 1.

3.3. Quantitative analysis for PC-OOH in human plasma

Quantitative analyses for PC-OOH were performed by LC/MS system with selected reaction monitoring (SRM) of the fragment ion at *m/z* 184.0 corresponding to choline phosphate in common with both standards (PC16:0/18:2-OOH and PC18:0/18:2-OOH) in positive ion mode. Fig. 3A and A′ shows the SRM chromatograms derived from the precursor ion at *m/z* 790.6 of the synthetic PC 16:0/18:2-OOH (0.6 pmol) and plasma extract, respectively. The peaks at 4.78 and 4.83 min (a) are observed in Fig. 3A and A′, so the peak (a) in Fig. 3A′ was identified as PC 16:0/18:2-OOH indicating its presence in plasma. From the peak shape, it was considered that the peak (a) may not be isomerically pure but the shoulder dose has not been interfered with the measurement of PC 16:0/18:2-OOH. There is a difference of 0.05 min in retention time, and that was maybe caused by two reasons. One is the different intensities between

Table 2
Significant ions of standards and plasma obtained from spectra by qualitative LC–MS system.

	MS (Precursor ions) $[M+H]^+$ (m/z)	MS/MS (product ions) $[H_2PO_4(CH_2)_2N(CH_3)_3]^+$
Synthetic PC 16:0/18:2-OOH	790.6	184.0
Plasma (a)	790.6	184.0
Synthetic PC 18:0/18:2-OOH	818.7	184.0
Plasma (b)	818.7	184.0
Synthetic PC 18:0/22:1-OOH (IS)	876.8	184.0

synthetic PC 16:0/18:2-OOH and endogenous PC 16:0/18:2-OOH in plasma; the other is that the LC system is not very stable. We have examined the changes of the retention time for PC 16:0/18:2-OOH as below. The synthetic PC 16:0/18:2-OOH (0.6 pmol) was analyzed with the quantitative LC/MS system for five times within 2 h, and the retention times showed little changes (range: 4.76–4.82 min). Fig. 3B and B′ shows the SRM chromatograms of the synthetic PC 18:0/18:2-OOH (0.6 pmol) and plasma extract, derived from the precursor ion at m/z 818.7, respectively; peaks at 4.84 and 4.86 min (b) were both identified as PC 18:0/18:2-OOH. Fig. 3C and C′ shows the SRM chromatograms of the IS (0.6 pmol) and plasma extract spiked with the IS (12.5 pmol), derived from the precursor ion at m/z 876.8. Both the IS and spiked sample showed identical peaks at 4.93 min as shown in Fig. 3C and C′, indicating that the IS (PC 18:0/22:1-OOH) is not subject to interference from endogenous components for the determination of PC-OOH in plasma. The precursor and product ions are summarized in Table 2.

3.4. Calibration curves of PC-OOH and concentration of PC-OOH in human plasma

Two calibration curves for PC 16:0/18:2-OOH and PC 18:0/18:2-OOH were constructed (Fig. 4). Data was plotted as the peak area ratios of PC 16:0/18:2-OOH (or PC 18:0/18:2-OOH) to the IS versus the amounts of PC 16:0/18:2-OOH (or PC 18:0/18:2-OOH). The curves showed good linearity throughout the range 0.1–1.0 pmol (PC 16:0/18:2-OOH, $y = 1.0533x + 0.0045$, $r^2 = 0.9989$; PC 18:0/18:2-OOH, $y = 1.3659x + 0.0173$, $r^2 = 0.9979$ where x is the amounts of hydroperoxide and y is the ratio about peak area of PC-OOH to that of the IS). The response factors relative to the IS are 0.634 and 0.843 for PC 16:0/18:2-OOH and PC 18:0/18:2-OOH, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were determined using synthetic standards. The LOD (S/N = 3:1) was 0.01 pmol, and the LOQ (S/N = 6:1) was 0.1 pmol for both of PC 16:0/18:2-OOH and PC 18:0/18:2-OOH.

The same method was applied to determination of PC-OOH concentrations in the plasma from the volunteer. The plasma concentrations of PC 16:0/18:2-OOH and PC 18:0/18:2-OOH were 89 and 32 nM, respectively.

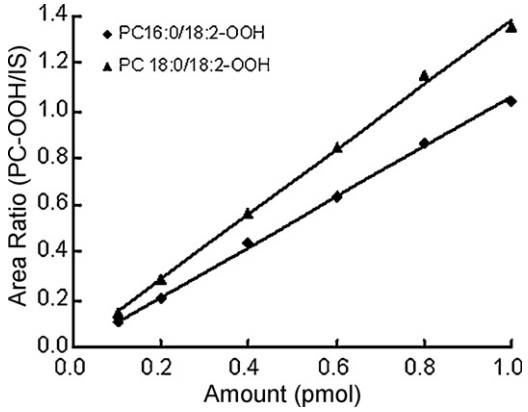

Fig. 4. The calibration curves of synthetic PC 16:0/18:2-OOH and PC 18:0/18:2-OOH constructed using different amount (0.1–1.0 pmol/injection). For abbreviation and LC/MS conditions see text.

4. Discussion

The main discoveries of this study were that (i) the hydroperoxide of PC 18:0/18:2 could be qualitatively identified in plasma by our qualitative LC/MS/MS and (ii) that the concentration of PC 16:0/18:2-OOH and PC 18:0/18:2-OOH in plasma could be quantified by LC/MS with SRM.

Using two synthetic standards (PC 16:0/18:2-OOH and PC 18:0/18:2-OOH), we have developed an unequivocal method for identification and quantification of PC 16:0/18:2-OOH and PC 18:0/18:2-OOH based on their mass spectral characteristics. Although PC 16:0/18:2-OOH has been identified previously [11], this study describes for the first time that PC 18:0/18:2-OOH has been identified and quantified in human plasma.

The success of the method, in significant part, is due to the use of a non-endogenous, synthetic internal standard (PC 18:0/22:1-OOH) that mimics the chemical, chromatographic and mass spectral characteristics of the target compounds. These characteristics allow the relative ratios about the two-targeted PC-OOH to be reliably iterated throughout the range 0.1–1.0 pmol which in turn provides for good linearity in the dynamic range necessary for the assaying of physiological specimens. We then applied the method to the determination of archetypal lipid peroxidation products in human plasma.

5. Conclusion

We were able to identify the species of PC-OOH using the qualitative LC/MS and LC/MS/MS in different configurations for the quantitative determination of PC 16:0/18:2-OOH and PC 18:0/18:2-OOH. Using this method we have successfully detected and quantified the concentrations of PC 16:0/18:2-OOH (89 nM) and PC 18:0/18:2-OOH (32 nM) in human plasma for the first time. These analytical methods are useful for studying biological samples, and will be helpful in clarifying incompletely understood pathway of various common diseases.

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References

[1] J.L. Witztum, D. Steinberg, J. Clin. Invest. 88 (1991) 1785.
[2] W.R. Markesbery, Free Radic. Biol. Med. 23 (1997) 134.
[3] A.N. Fonteh, R.J. Harrington, A.F. Huhmer, R.G. Biringer, J.N. Riggins, M.G. Harrington, Dis. Markers 22 (2006) 39.
[4] S.-P. Hui, T. Yoshimura, T. Murai, H. Chiba, T. Kurosawa, Anal. Sci. 16 (2000) 1023.
[5] S.-P. Hui, T. Murai, T. Yoshimura, H. Chiba, T. Kurosawa, Lipids 38 (2003) 1287.
[6] S.-P. Hui, T. Murai, T. Yoshimura, H. Chiba, H. Nagasaka, T. Kurosawa, Lipids 40 (2005) 515.

[7] T. Miyazawa, K. Yasuda, K. Fujimoto, T. Kaneda, J. Biochem. 103 (1988) 744.
[8] S.-P. Hui, H. Chiba, T. Sakurai, C. Asakawa, H. Nagasaka, T. Murai, H. Ide, T. Kurosawa, J. Chromatogr. B 857 (2007) 158.
[9] A. Reis, P. Domingues, A.J. Ferrer-Correia, M.R. Domingues, Rapid Commun. Mass Spectrom. 18 (2004) 2849.
[10] M. Ishida, T. Yamazaki, T. Houjou, M. Imagawa, A. Harada, K. Inoue, R. Taguchi, Rapid Commun. Mass Spectrom. 18 (2004) 2486.
[11] J. Adachi, N. Yoshioka, R. Funae, Y. Nagasaki, T. Naito, Y. Ueno, Lipids 39 (2004) 891.
[12] A. Reis, M.R. Domingues, F.M. Amado, A.J. Ferrer-Correia, P.D. Omingues, Biomed. Chromatogr. 19 (2005) 129.
[13] H. Nakanishi, Y. Iida, T. Shimizu, R. Taguchi, J. Chromatogr. B 877 (2009) 1366.
[14] M.R. Domingues, C. Simoes, J.P. da Costa, A. Reis, P. Domingues, Biomed. Chromatogr. 23 (2009) 588.