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Separations of molecular species of phosphatidic acid by high-performance liquid chromatography

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Abstract

Molecular species of phosphatidic acid (PA) were separated by reversed-phase high-performance liquid chromatography with mobile phases of acetonitrile–methanol–water containing quaternary ammonium phosphates (QAPs). Effective resolution of PA complexes was achieved by using low-mass QAPs at concentrations ≥ 50 mM or high-mass QAPs at concentrations ≥ 10 mM. Capacity factor (k') values were found to be dramatically influenced by the type and concentration of QAP, stationary phase specifications, and mobile phase solvent compositions. An increase in the acetonitrile–methanol ratio of the mobile phase tended to enhance the retention and separation of the polar lipid components. Correlation of logarithmic k' values with the total number of carbon atoms in QAP appeared to yield non-linear relationships. Compositions of major molecular species in various PA samples were determined by calibration with synthetic PA standards. Distribution patterns of PA molecular species in samples derived from animal and plant sources were compared.

1. Introduction

Clusters of phosphatidic acid (PA) (Fig. 1) are negatively charged polar lipids occurring in small amounts in cell membranes of animals and plants. They are important metabolic intermediates of phospholipid (PL) biosyntheses. Because of high activity of plant phospholipases, PA is known to be the major breakdown product of various PL classes in plants through specific enzymatic hydrolysis by phospholipase D. Several studies [1–4] show that high levels of PA has been found in PL fractions of soybeans stored for extended periods of time, elevated temperatures, and high moisture contents. Degummed soybean oils contain predominantly PA in the polar lipid isolates [4–7]. Our current research on the assessment of the impact of genetic modifications on oil quality required development of reliable, sensitive chromatographic techniques for analyses of PL classes and their molecular species. Normally, the polar lipid fractions of soybeans comprise primarily phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and PA. Of these, PA amounts to ca. 5% of the total PL in the standard crude oils. Consequently, the low abundance of PA in oilseeds necessitates investigations of chromatographic methodology that would provide assay procedures for sensitive measurements of PA species.

The literature information on the separation

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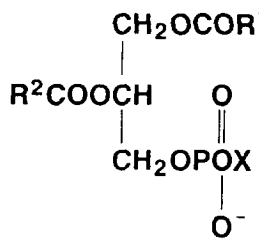

X = H, PA
X = Glycerol, PG
X = Inositol, PI
X = Serine, PS
X = PG, DPG

Fig. 1. General structure of negatively charged phospholipids. R^1 and R^2 represent alkyl or alkenyl groups of fatty acid chains.

and quantification of PA molecular species is sparse in contrast to the vast volume of available HPLC methods for the analysis of the major soybean PL (PE, PI and PC). Recently, certain derivatives of PA subcomponents have been separated by reversed-phase high-performance liquid chromatography (HPLC) [8–14]. In all of the published methods, the PA analytes are first converted to their dimethyl esters [8–13], dibenzyl esters [10] or monomethyl esters [14] prior to HPLC analyses. Due to the highly polar nature of PA molecules, HPLC speciation of molecular species of intact underivatized PA complexes has met with difficulties. Earlier attempts to resolve PA complexes failed, even though HPLC conditions employed were identical to those under which the corresponding species of monomethyl esters of PA were well separated [14]. Since direct HPLC separations of intact PA subcomponents are not known, this paper reports the results of a reversed-phase HPLC study on the baseline separation of the molecular species of intact PA molecules.

2. Experimental

2.1. Chemicals and reagents

Egg- and soybean PA were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Wheat germ- and bovine brain PA were obtained from Matreya (Pleasant Gap, PA, USA). Degummed and modified soybean PA were prepared from respective oils following procedures as described previously [4,15]. Synthetic PA standards were obtained either from Avanti Polar Lipids or Sigma (St. Louis, MO, USA). All commercial natural PA samples were prepared from corresponding natural PC by transphosphatidylation with phospholipase D. Alkyltriethylammonium phosphates (ATEAP) and tetrabutylammonium phosphate (TBAP) were obtained from Regis (Morton Grove, IL, USA). Most of the symmetrically substituted tetraalkylammonium phosphates (TAAP) (carbon chains C_1 to C_3) were prepared from the corresponding hydroxides (Aldrich, Milwaukee, WI, USA) by titration with 85% phosphoric acid (Fisher Chemicals, Fair Lawn, NJ, USA) until desired pH values were obtained. HPLC-grade acetonitrile and methanol were products of EM Separations (Gibbstown, NJ, USA). Ultra-pure HPLC water was obtained by filtering through a Waters–Millipore (Milford, MA, USA) Milli-Q water purifier.

2.2. High-performance liquid chromatography

All HPLC experiments were performed on a Spectra-Physics (Thermo Separations, San Jose, CA, USA) liquid chromatograph Model SP 8700 solvent-delivery system. The LC unit was interfaced with an LDC Analytical (Riviera Beach, FL, USA) SpectroMonitor D multiple-wavelength UV detector. The polar lipid analytes were detected at a wavelength of 208 nm. Detector signals were recorded with an OminiScribe recorder (Houston Instruments, Houston, TX, USA). Mobile phases consisted of acetonitrile, methanol, water and quaternary ammonium phosphates (QAPs). In optimization experiments, QAP concentrations in the range 5–50 m*M* were employed. The HPLC eluents, prepared freshly before assays, were filtered, degassed, and pumped isocratically through a reversed-phase column at a flow-rate of 1 ml/min unless specified otherwise.

Analytical samples in aliquots of 0.5–5 μl 1% solutions were injected onto a column via a

Rheodyne (Cotati, CA, USA) Model 7125 injector equipped with a 10-μl loop. Three replicate injections were made for all sample analyses. Samples were stored in amber vials in a freezer at −30°C whenever not in use. Stationary phases were obtained from different commercial suppliers: (1) Ultrasphere C_{18}, 5 μm, 250 (or 150) × 4.6 mm I.D. (Beckman Instruments, San Ramon, CA, USA), (2) NovaPak C_{18}, 4 μm, 300 × 3.9 mm I.D. (Waters Chromatography, Milford, MA, USA), (3) Adsorbsphere HS C_{18}, 5 μm, 250 × 4.6 mm I.D. (Alltech, Deerfield, IL, USA), (4) YMC-Pak ODS-A, 5 μm, 250 × 4.6 mm I.D. (YMC, Wilmington, NC, USA), and (5) LiChrospher RP-18, 3 μm, 250 × 4 mm I.D. (EM Separations).

For confirmation purposes, individual HPLC peaks attributable to PA molecular species were collected and subjected to fatty acid analyses by a published HCl–methanol/gas chromatographic procedure [16]. Fatty acids were characterized as methyl esters using a Varian Model 3400 gas chromatograph equipped with a flame ionization detector. Samples were injected onto a 30 m × 0.25 mm fused-silica capillary column coated with 0.2 μm SP 2330 (Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas. The column temperature was programmed from 200 to 220°C at 10°C/min after an initial hold of 15 min.

3. Results and discussion

In addition to the four major PL classes, there are phosphatidylserine (PS), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) present in soybeans as minor PL constituents. The majority of these soybean PLs are negatively-charged compounds (PA, PG, PI, PS and DPG) as depicted in Fig. 1. Neutralization of the negative charges by cationic QAP counter ions in mobile phases should facilitate differential partition between the PL solutes and the hydrocarbonaceous phase in reversed-phase HPLC systems. With the exception of PA, molecular species of the negatively charged soybean lipids including N-derivatives of PE have recently been separated by reversed-phase ion-pair HPLC [17–21].

Examination of the PA structure (Fig. 1) indicates that the doubly anionic PA molecules devoid of hydrocarbonaceous functionality in the head group are relatively less hydrophobic than other negatively charged PL compounds found in soybeans. The mobile phase conditions used in HPLC of the more hydrophobic negatively-charged PLs [17–21] are apparently inadequate to confer a certain degree of hydrophobicity required for solvophobic interactions in the reversed-phase separation processes. Therefore, the strategy of increasing the concentration of QAP or using high-molecular-mass QAPs proved fruitful and led to the first separation of the intact PA molecular species. This direct approach to the analysis of intact PA species is advantageous over the existing chemical derivatization methods. Since the latter methods entail structural modifications, the molecular integrity of the analytes may not be preserved under drastic derivatization conditions.

Fig. 2 shows typical separations of PA molecular species under various HPLC conditions. As observed in HPLC of other negatively charged polar lipids [17–21], no appreciable retention and resolution of PA molecular species were observed in initial exploratory experiments where no QAP electrolytes were used in mobile phases. HPLC of a mixture of PA components on an octadecylsilica (ODS) column with a mobile phase of acetonitrile–methanol–water containing a QAP at a low concentration (0.5–5 mM) produced a broad band with some peak tailing [14] or, at best, ill-defined multiple bands (Fig. 2A). Adding more QAP to the mobile phases resulted in near-baseline separations of the PA molecular species as illustrated in Fig. 2B–D.

Retention data in Table 1 clearly demonstrate that the six major egg PA components were more strongly retained (higher k' values) by octadecylsilica (ODS) in mobile phases containing higher concentrations of QAP counter-ions. In the presence of increased concentrations of the cationic buffers, the anionic characteristics of the analytes appeared to be neutralized to further extent by the counter-ions, enhancing hy-

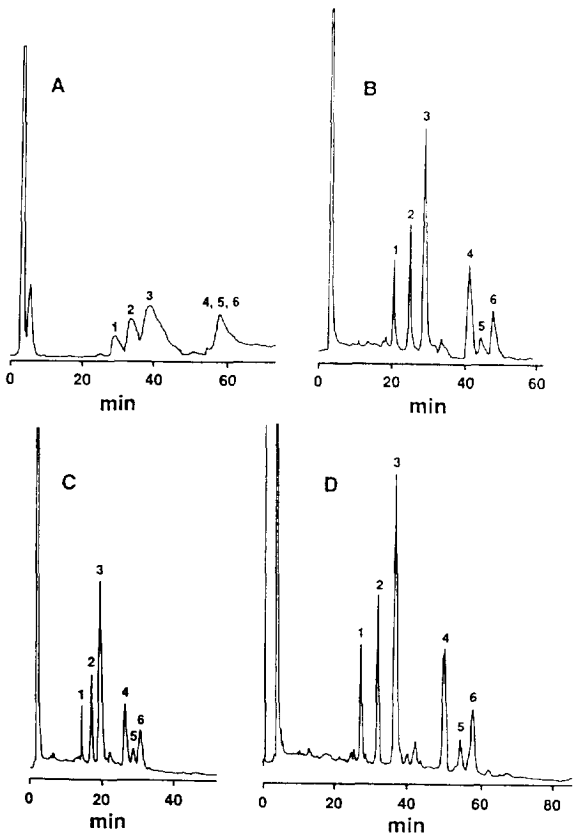

Fig. 2. HPLC separations of egg PA components. Columns: Beckman Ultrasphere C_{18}, 250 × 4.6 mm I.D. (A, B), or 150 × 4.6 mm I.D. (C); YMC-ODS (D). Mobile phases: acetonitrile–methanol–water (70:22:8) containing 5 m*M* PTAP at pH 6.5 (A); acetonitrile–methanol–water (70:26:4) containing 20 m*M* TBAP at pH 6.5 (B); acetonitrile–methanol–water (49:49:2) containing 50 m*M* TMAP at pH 6.5 (C, D). Peaks: 1 = 16:0–22:6; 2 = 16:0–20:4; 3 = 16:0–18:2; 4 = 16:0–18:1, 5 = 18:0–20:4 + 18:1–18:1; 6 = 18:0–18:2.

drophobic interactions in the reversed-phase chromatographic processes. The counter-ion concentration effects were more pronounced in HPLC experiments with higher water contents and heavier QAP in mobile phases [Table 1, conditions A1 (HTAP) *vs.* conditions A2 (OTAP)], which partly reflected the effect of the change in QAP types. On the other hand, the differences in k' values between the corresponding PA components were relatively small, when the retention data obtained with 25 m*M* TMAP were compared with those obtained with 50 m*M* TMAP (Table 1, conditions B). The positive concentration effects (k' values increase with increasing QAP concentrations) were found independent of the type of stationary phases evaluated (Table 1, conditions A *vs.* B), as observed in the HPLC studies on other soybean PL classes [17–21].

Two structural series of QAPs were chosen for study to determine if the retention behavior of the resolved PA components would be affected by incorporation of different types of QAP counter-ions in mobile phases. The tetraalkylammonium phosphate (TAAP) series includes four symmetrically substituted alkyl-ammonium phosphates: tetramethylammonium phosphate (TMAP), tetraethylammonium phosphate (TEAP), tetrapropylammonium phosphate (TPAP) and tetrabutylammonium phosphate (TBAP). The alkyltriethylammonium phosphate (ATEAP) series includes four unsymmetrically substituted ammonium phosphates: pentyltriethylammonium phosphate (PTAP), heptyltriethylammonium phosphate (HTAP), octyltriethylammonium phosphate (OTAP) and dodecyltriethylammonium phosphate (DTAP). Table 2 shows examples of structural effects of QAPs on the retention characteristics (k' values) of PA molecular species. As expected, greater k' values of the analyte components were observed in HPLC with QAP of higher molecular mass. In other words, PA analytes had longer retention times in mobile phases containing a higher-molecular-mass member of QAP in both structural series (Table 2 and Fig. 3).

Attempts to correlate the total number (n) of carbon atoms in QAP with logarithmic k' values failed to produce straight lines. The correlation results seems to be in variance with the linear relationships observed previously in HPLC of all other anionic polar lipids [17–21]. The $\ln k'$–n plots in Fig. 3 exhibit two sets of curves with marked differences in curvature. Evidently, changes in the magnitude of hydrophobicity (a function of n) of the QAP electrolyte caused greater responses in k' values for the ATEAP series than for the TAAP series.

Inspection of the HPLC data in Table 3 indicates that k' values and separation factors

Table 1
Concentration effects of quaternary ammonium phosphates on capacity factors, k', of molecular species of egg PA derived from egg PC

HPLC conditions[a]	QAP	QAP concentration (mM), pH 6.5	Capacity factor, k'					
			Component 1	Component 2	Component 3	Component 4	Component 5	Component 6
A1	TMAP	10.00	2.50	2.91	3.30	4.67	5.00	5.41
		50.00	6.48	7.75	8.90	12.2	13.0	13.8
	HTAP	5.00	2.19	2.58	3.22	4.50	4.85	5.14
		10.00	2.52	3.06	3.67	4.95	5.42	5.81
A2	OTAP	5.00	9.67	11.3	12.7	19.5	24.6	40.8
		10.00	16.8	20.3	22.9	36.3	43.2	69.3
B	TMAP	25.0	4.34	5.51	6.92	9.00	9.99	11.2
		47.7	4.67	5.75	7.15	9.33	10.2	11.5

TMAP = Tetramethylammonium phosphate; HTAP = heptyltriethylammonium phosphate; OTAP = octyltriethylammonium phosphate.
[a] HPLC conditions: A1 = mobile phase acetonitrile–methanol–water (49:49:2), pH 6.50; Beckman Ultrasphere C_{18} column, 250 × 4.6 mm I.D.; A2 = mobile phase acetonitrile–methanol–water (70:22:8), pH 7.50; column as A1; B = mobile phase acetonitrile–methanol–water (70:28:2), pH 7.50; column NovaPak 18, 300 × 3.9 mm I.D.

(α) for adjacent components were dramatically influenced by the variation in solvent compositions of mobile phases. As described in the Experimental section, the mobile phase solvents employed acetonitrile, methanol and water throughout the study. Few separations (α = 1.00) of the lipid species were achieved when acetonitrile was not used in mobile phases (Table 3,

Table 2
Effects of the type of quaternary ammonium phosphates on the capacity factors, k', of molecular species of egg PA derived from egg PC

QAP (n)[a]	Capacity factor, k'					
	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6
TAAP series						
TMAP (4)	2.48	2.89	3.29	4.64	4.95	5.37
TEAP (8)	2.53	3.00	3.44	4.77	5.10	5.53
TPAP (12)	2.69	3.24	3.74	5.10	5.47	5.93
TBAP (16)	3.00	3.66	4.17	5.65	6.02	6.50
ATEAP series						
PTAP (11)	2.17	2.58	3.09	4.16	4.50	4.91
HTAP (13)	2.49	3.06	3.67	4.95	5.42	5.81
OTAP (14)	2.83	3.49	4.18	5.69	6.11	6.55
DTAP (18)	7.84	10.3	11.7	15.9	16.8	18.5

HPLC conditions: mobile phase, acetonitrile–methanol–water (49:49:2) containing 10 mM QAP at pH 6.5; stationary phase, Beckman Ultrasphere C_{18}. TAAP = Tetraalkylammonium phosphate; TMAP = tetramethylammonium phosphate; TEAP = tetraethylammonium phosphate; TPAP = tetrapropylammonium phosphate; TBAP = tetrabutylammonium phosphate; ATEAP = alkyltriethylammonium phosphate; PTAP = pentyltriethylammonium phosphate; HTAP = heptyltriethylammonium phosphate; OTAP = octyltriethylammonium phosphate; DTAP = dodecyltriethylammonium phosphate.
[a] n = Total number of carbon atoms in QAP.

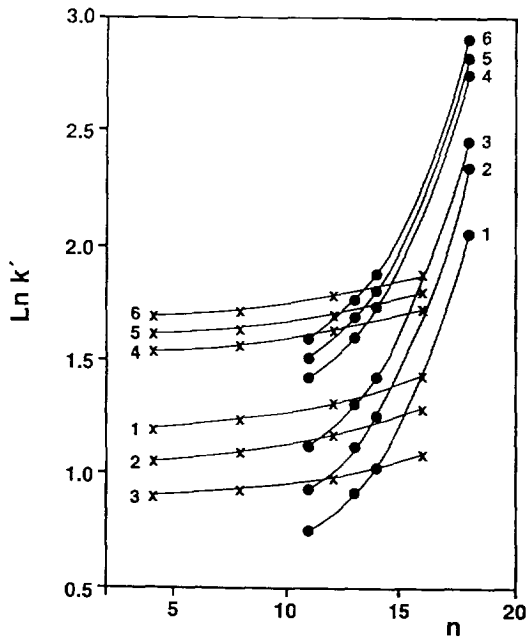

Fig. 3. Non-linear relation between ln k' of PA components and the total number (n) of carbon atoms in QAP. Curves with dots and crosses represent experiments with ATEAP and TAAP, respectively. HPLC conditions, column: Beckman Ultrasphere C_{18}, 250 × 4.6 mm I.D.; mobile phase: acetonitrile–methanol–water (49:49:2) containing 10 mM QAP at pH 6.5. For component identification, see Fig. 2.

conditions A). The k' and α values had the tendency to increase with increasing proportions of acetonitrile in acetonitrile–methanol–water mobile phases. Conversely, the presence of high methanol content in mobile phases had an adverse effect on component separations, as evidenced by low α values (Table 3, conditions A and B).

In consideration of the acidic properties of PA molecules, it was worthwhile to conduct a pH study to probe the effect of mobile phase pH on their retention characteristics as measured by the k' values. Generally, at a higher mobile phase pH value, the PA analytes were less retained (lower k' values) on the ODS phase (Table 4). In addition, notable enhancement in the pH effect was manifested in experiments with high water contents of mobile phases (Table 4, B). The inverse relationship between pH values and k' values of PA analytes may be explained in terms of an ion suppression rationale. The results of this pH study are parallel to those described in an earlier work [14] in which phosphoric acid and ammonia solutions were added to mobile phases and a polystyrene–divinylbenzene resin column was used.

A number of commercial ODS stationary

Table 3
Effects of the mobile phase solvent composition on the capacity factors, k', of molecular species of egg PA derived from egg PC

Conditions	Mobile phase solvent ratio ACN–MeOH–water	Component 1		Component 2		Component 3		Component 4		Component 5		Component 6
		k'	α	k'	α	k'	α	k'	α	k'	α	k'
A	49:49:2	3.38	(1.18)	4.00	(1.19)	4.75	(1.27)	6.05	(1.16)	6.99	(1.00)	6.99
	0:98:2	3.95	(1.00)	3.95	(1.00)	3.95	(1.33)	5.25	(1.00)	5.25	(1.00)	5.25
B	64:34:2	7.67	(1.22)	9.33	(1.20)	11.2	(1.38)	15.4	(1.14)	17.5	(1.33)	18.4
	49:49:2	6.48	(1.20)	7.75	(1.15)	8.90	(1.37)	12.2	(1.07)	13.0	(1.06)	13.8
C	70:22:8	15.5	(1.21)	18.7	(1.14)	21.3	(1.58)	33.6	(1.18)	39.5	(1.18)	46.5
	49:49:2	3.00	(1.22)	3.66	(1.14)	4.17	(1.35)	5.65	(1.07)	6.02	(1.08)	6.50
D	70:26:4	7.41	(1.23)	9.10	(1.19)	10.8	(1.45)	15.7	(1.10)	17.2	(1.09)	18.8
	49:49:2	5.58	(1.16)	6.50	(1.16)	7.51	(1.33)	10.0	(1.07)	10.7	(1.08)	11.6

HPLC conditions: mobile phases contained (A, B, D) 50 mM tetramethylammonium phosphate and (C) 10 mM tetrabutylammonium phosphate at pH values of (A, D) 7.50 and (B, C) 6.50; stationary phases, (A) Beckman Ultrasphere C_{18} (150 × 4.6 mm I.D.) and (B, C, D) Beckman Ultrasphere C_{18} (250 × 4.6 mm I.D.). ACN = Acetonitrile.

Table 4
Effects of the mobile phase pH on the capacity factors, k', of molecular species of egg PA derived from egg PC

Mobile phase pH	Capacity factor, k'					
	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6
(A) Acetonitrile–methanol–water (49:49:2), 50 mM TMAP						
7.50	5.58	6.50	7.51	10.0	10.7	11.6
6.50	6.48	7.75	8.90	12.2	13.0	13.8
(B) Acetonitrile–methanol–water (70:22:8), 10 mM TBAP						
7.50	8.00	9.58	10.8	16.7	19.5	20.5
6.50	15.5	18.7	21.3	33.6	39.5	46.5

HPLC conditions: stationary phase, Beckman Ultrasphere C_{18} column (250 × 4.6 mm I.D.)

phases were studied under identical HPLC conditions to evaluate their separation potential for the PA species interest. The data in Table 5 indicate that, with the exceptions of experiments C and E, variations in α and k' values among the ODS columns studied are fairly small. In view of the higher degree of hydrocarbonaceous surface coverage (20% carbon loading and 3.27 μmol/m^2 bonded phase) in Adsorbsphere ODS with 60 Å pore size, reversed-phase HPLC of PA on this stationary phase would be expected to lead to separations of components with highest k' values. However, the experimental results showed otherwise (Table 5, D). In fact, the use of a YMC ODS phase gave longest retention times (higher k' values) of the PA components as noted in experiment C of Table 5, presumably due to the relatively large pore size (120 Å) of the column packings. A divinylbenzene polymer-based ODS column (Table 5, E) showed lower selectivity (α values = 1.00 for the adjacent peaks 4, 5 and 6) for most of the PA species than a silica-based ODS phase (Table 5, F).

Table 6 shows the calibration data for six commercially available synthetic PA standards. Plotting the injected amounts of individual PA compounds against peak areas yielded linear correlation lines with slope values (m) ranging 0.088–8.005 cm^2/μg. Coefficients of variation in peak measurements and correlation coefficients averaged 2.82% and 0.9987, respectively. The amounts of PA components in unknown samples can be determined by the equation $Y = mX$, where Y and X represent the respective peak

Table 5
Stationary phase effects on the capacity factors, k', of molecular species of egg PA derived from egg PC

Stationary phase	Component 1		Component 2		Component 3		Component 4		Component 5		Component 6
	k'	α	k'	α	k'	α	k'	α	k'	α	k'
A	6.48	(1.20)	7.75	(1.15)	8.90	(1.37)	12.2	(1.07)	13.0	(1.06)	13.8
B	5.83	(1.20)	7.00	(1.19)	8.33	(1.33)	11.1	(1.15)	12.8	(1.04)	13.3
C	10.3	(1.19)	12.3	(1.17)	14.4	(1.35)	19.5	(1.10)	21.5	(1.07)	23.0
D	5.27	(1.20)	6.33	(1.20)	7.60	(1.34)	10.2	(1.13)	11.5	(1.06)	12.2
E	2.50	(1.10)	2.75	(1.05)	2.88	(1.98)	5.69	(1.00)	5.69	(1.00)	5.69
F	7.46	(1.20)	8.97	(1.16)	10.4	(1.41)	14.7	(1.08)	15.9	(1.08)	17.1

HPLC conditions: mobile phase, acetonitrile–methanol–water (49:49:2) containing 50 mM TMAP at pH 6.5; stationary phases: (A) Beckman Ultrasphere C_{18} (250 × 4.6 mm I.D.), (B) EM Separations LiChrospher RP-18 (250 × 4 mm I.D.), (C) YMC-ODS-A (250 × 4.6 mm I.D.), (D) Alltech Adsorbsphere HS C_{18} (250 × 4.6 mm I.D.), (E) AsahiPak ODP-50 (150 × 4.6 mm I.D.), (F) Beckman Ultrasphere C_{18} (150 × 4.6 mm I.D.). Flow-rates: (A–D) 1 ml/min, (E, F) 0.5 ml/min.

Table 6
Linear correlation between peak areas and injected amounts of selected synthetic PA species on a Beckman Ultrasphere C_{18} column (250 × 4.6 mm I.D.) in HPLC with a mobile phase of acetonitrile–methanol–water (49:49:2) containing 50 m*M* TMAP at pH 6.5

PA species	Slope (m) $cm^2/\mu g$	R.S.D. (%)	Correlation coefficient (r)
18:0–20:4-PA	8.005	2.83	0.9998
16:0–20:4-PA	4.804	2.76	0.9999
18:2–18:2-PA	2.399	2.40	0.9998
16:0–18:2-PA	1.682	2.71	0.9979
18:1–18:1-PA	0.392	3.02	0.9966
16:0–18:1-PA	0.088	3.20	0.9980

Calibration lines expressed by $Y = mX$, where X, Y and m represent the amount injected, the peak area and the slope, respectively. Relative standard deviations (R.S.D.s) are based on mean values of three determinations of peak areas.

area and amount of a PA species present in a sample. The calibration equation implies that all the curves crossed the origin at zero. In light of the different degree of UV absorptivity among the PA molecular species analyzed, construction of calibration plots is an indispensable procedure for the quantitative analysis of the polar lipids by HPLC–UV detection [15].

With the aid of the calibration data in Table 6, compositions of the major PA components in some PA samples derived from various commercial sources were determined. The results are summarized in Table 7. The PA components were identified by peak matching with synthetic PA standards and by fatty acid analyses. Similar to the findings in a previous HPLC study on the separation of diverse groups of PL subcomponents [22], the distribution patterns of PA molecular species in samples of animal origins are generally more complex than those derived from plants (Fig. 4A–C *vs.* Fig. 4D and E). The chromatographic profile of a crude sample of degummed soybean oil revealed extra peaks due to contamination by impurities presumably derived from other PL species (Fig. 4C).

In conclusion, the results of this study represent the first report on the reversed-phase HPLC separation of molecular species of intact PA without derivatization. Using the HPLC–UV detection technique developed, quantitative analyses of the PA samples can be achieved by calibration with individual synthetic standards. The essence of attaining satisfactory component resolution lies in the modification of the polar anionic PA solutes with QAP cationic electrolytes in the mobile phase systems for strengthening hydrophobic interactions during

Table 7
Determination of the composition of major PA molecular species in animal and plant samples

Molecular species	Composition (%)				
	Egg	Brain	Soybeans	Soybeans (degummed)	Wheat
18:3–18:3	ND	ND	4.52	4.07	6.33
18:2–18:3	ND	ND	12.7	10.4	17.9
18:1–18:2	ND	ND	6.79	6.37	5.09
18:0–20:4	3.94	11.3	ND	ND	ND
16:0–20:4	3.35	3.18	ND	ND	ND
18:2–18:2	ND	ND	43.4	31.7	36.6
16:0–18:2	19.1	7.31	18.1	21.3	9.94
18:1–18:1	3.00	ND	ND	ND	ND
16:0–18:1	43.6	37.6	TR	TR	TR
18:0–18:2	9.37	4.52	3.60	3.09	2.70
18:0–18:1	16.8	13.8	ND	ND	ND
Others	0.84	22.3	10.9	23.1	21.4

HPLC conditions were same as in Table 6. ND = None detected; TR = trace.

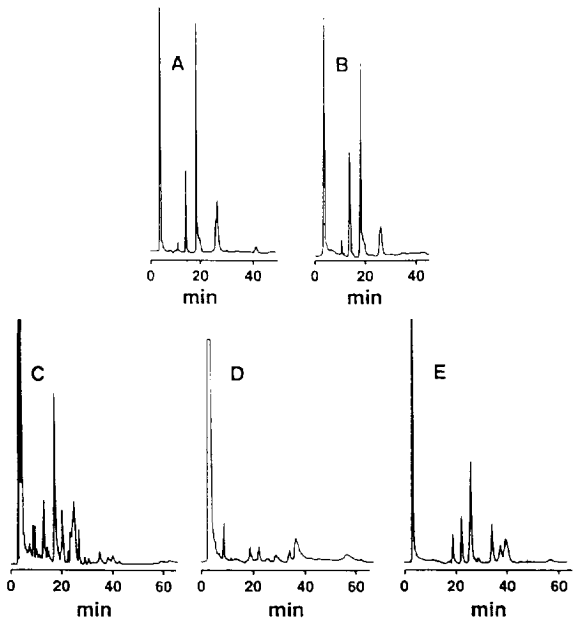

Fig. 4. HPLC separations of PA components in samples derived from animals and plants. (A) Soybeans, (B) wheat germ, (C) degummed soybean oil, crude sample, (D) bovine brain, (E) egg. HPLC conditions, column: Beckman Ultrasphere C_{18}; mobile phase as in Fig. 2C and D.

the separation processes. Thus, mobile phases in reversed-phase HPLC of the doubly charged PA require more hydrocarbonaceous electrolyte additives than those of singly charged PL. Optimization of HPLC conditions in the context of mobile phase variables and column specifications facilitates baseline resolution of the PA components with reasonable retention times. The method may be applicable to the characterization of PA molecular species in a wide range of biological and physiological sample matrices derived from cell membranes of animals and plants.

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