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Reversed-phase separations of nitrogenous phospholipids on an octadecanoyl poly(vinyl alcohol) phase[1]

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Abstract

Molecular species of nitrogenous phospholipids (PLs) phosphatidylcholine (PC), phosphatidylethanolamine (PE), PE-derivatives and sphingomyelin (SP) were separated on an octadecanoyl poly(vinyl alcohol) (ODPVA) column by reversed-phase HPLC with UV and evaporative light scattering detection (ELSD). Mobile phases employed variable proportions of acetonitrile, methanol and water. HPLC–UV of the polar lipids yielded components with peak intensities somewhat different from those obtained by HPLC–ELSD despite discernible similarity in the peak profiles observed in the two detection systems. Incorporation of ammonium hydroxide in mobile phases resulted in a decrease in analyte retention. The mobile phase basicity effect on capacity factors of PE species was significantly greater than that of PC counterparts. The new ODPVA HPLC–ELSD technique was applied to the analysis of PC and PE molecular species in vegetable oils. © 1997 Elsevier Science B.V.

Keywords: Stationary phases, LC; Octadecanoyl poly(vinyl alcohol) stationary phase; Phospholipids; Phosphatidylcholine; Phosphatidylethanolamine; Sphingomyelin; Lipids

1. Introduction

Phospholipids (PLs) are important cellular constituents of animals and plants. Physicochemical properties of cell membranes are dependent upon the composition of PL classes and subclasses. Compositional variations of the polar lipids are also known to have significant influence on the cellular biochemical processes. In light of the close relation between oil quality and plant constituents, quantification of PLs in oilseeds provides valuable information on their distributions in vegetable oils. In addition, accurate and rapid analytical methods for PL measurements have been of continuous demand for lipid scientists.

Because of the presence of basic amino groups and ionic charges in the title PL compounds, separations of their molecular species by conventional reversed-phase high-performance liquid chromatography (HPLC) with octadecylsilica (ODS) phases have met with definitive shortcomings: (i) large amounts of samples needed for analysis, (ii) peak broadening and tailing, (iii) long analysis times, (iv) low detection sensitivity, (v) mobile phase buffer additives required, (vi) working pH range limited to

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the 2–7 range and (vii) volatile toxic organic solvents used. To dissolve the problems associated with the conventional reversed-phase HPLC practice, lipid analysts have used buffered mobile phases in conjunction with deactivated silica columns for the quantification of PL molecular species.

For many years, alkyl-bonded silica columns have been extensively used in the reversed-phase HPLC analysis of neutral PLs [1–10]. Recently, Christie's group pioneered the separation of PL molecular species on a polystyrene–divinylbenzene column [11]. The scarcity of literature information on the application of nonsilica-based stationary phases in PL separations provided impetus to undertake this study. In connection with another study on minor oil constituents [12], it was shown that an octadecanoyl poly(vinyl alcohol) (ODPVA) phase exhibited unusual selectivity for certain closely related isomeric compounds. In view of additional advantages of using the ODPVA column for its inertness toward basic media and unique amiability with basic compounds, we studied the separation of the title PL compounds on this polymer phase in the reversed-phase mode.

2. Experimental

2.1. Chemicals and reagents

Selected individual PL classes phosphatidylcholine (PC), phosphatidylethanolamine (PE), PE derivatives and sphingomyelin (SP) derived from plant and animal sources were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Egg PE and its N-alkyl and N-acylamine analogues derived from egg PC were also obtained from Avanti Polar Lipids (Avanti prepared the egg PE derivatives by initial transphosphatidylation of egg PC followed by N-acylation of the product, egg PE). Various oilseeds were supplied by Cargill, (Minneapolis, MN, USA) and Calgene Inc. (Davis, CA, USA). Canola PC and PE samples were prepared from the oilseeds according to published procedures [13–15]. Hexane extracts of the oilseeds were first chromatographed on a silica gel column which was sequentially eluted with chloroform, acetone, methanol and methanol containing 0.1% phosphoric acid. The latter two methanolic eluates were pooled for recovery of total PLs. Preparative HPLC of the polar lipid isolates with mobile phases of chloroform–methyl *tert.*-butyl ether–methanol–ammonium hydroxide under gradient elution [13–15] yielded major fractions of PC and PE. These individually purified samples were used in all assays. All the samples analyzed for molecular species contained practically single PL class.

HPLC solvents acetonitrile and ammonium hydroxide (as a 28.0–30.0% ammonia solution) were purchased from EM Separations (Gibbstown, NJ, USA). HPLC methanol and other unspecified reagents were obtained from Fisher Chemicals (Fair Lawn, NJ, USA). HPLC-grade water was obtained by treating distilled water with a Milli-Q water purifier (Millipore, Bedford, MA, USA).

2.2. High-performance liquid chromatography

In all experiments, a Thermo Separation Products (Fremont, CA, USA) Model 8700 liquid chromatograph equipped with LDC Analytical (Riviera Beach, FL, USA) SpectroMonitor D UV detector was used. PL molecular species were detected at 208 nm. In some experiments, the LC solvent delivery system was interfaced with an Alltech Mark III evaporative light scattering detection (ELSD) system. Mobile phases containing variable proportions of acetonitrile–methanol–water were degassed with helium sparge, filtered through a 0.2-μm filter, and then pumped onto a reversed-phase column at a flow-rate of 1 ml/min.

For comparative studies, numerous commercial reversed-phase HPLC columns (5 μm, 250×4.6 mm I.D.) were evaluated in addition to the ODPVA column: (1) Astec (Whippany, NJ, USA) AsahiPak ODP with octadecanoyl poly(vinyl alcohol) (ODPVA) packings, (2) Alltech (Deerfield, IL, USA) HEMA-RP with octadecyl hydroxy ethyl methacrylate polymer packings (10 μm), (3) Jordi (Bellingham, MA, USA) C_{18}-DVB with divinylbenzene polymer packings, (4) Polymer Laboratories (Amherst, MA, USA) PLRP-S with polystyrene divinylbenzene packings, (5) Phenomenex (Torrance, CA, USA) Prodigy ODS-2 and (6) YMC (Wilmington, NC, USA) ODS-A. Most satisfactory results were obtained with the ODPVA phase.

Aliquots (1–5 μl) of freshly prepared samples in methanol (0.1–5 mg/ml) were injected onto a HPLC

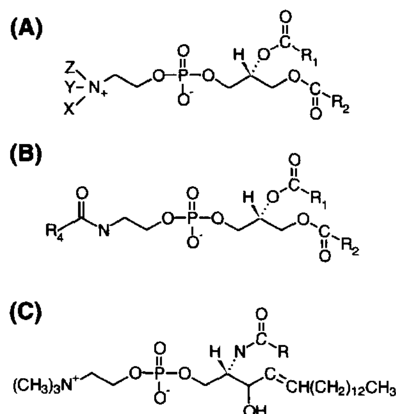

Fig. 1. Structures of investigated glycerophospholipids (GPLs) and sphingophospholipids (SPLs) where R_1, and R_2 represent respective alkenyl and alkyl groups of fatty acid chains of GPLs, and R represents alkenyl or alkyl group of the fatty acid chain of SPLs. Compound abbreviations: (A) PE, X=Y=Z=H; MMPE, X=Y=H, Z=CH_3; DMPE, X=Y=CH_3, Z=H; PC, X=Y=Z=CH_3. (B) CAPE, R_4=aminopentyl; DAPE, R_4=aminoundecyl. (C) SP.

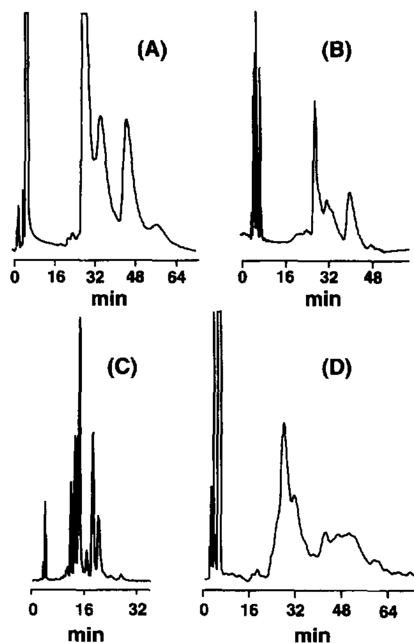

Fig. 2. Reversed-phase HPLC–UV separations of egg PC on various polymer phases: (A) PLRP-S, (B) C_{18}-DVB, (C) ODPVA, (D) HEMA-RP. Mobile phases: (A,B) acetonitrile–MeOH–water (45:45:10); (C) acetonitrile–MeOH–water (47.5:47.5:5); (D) acetonitrile–MeOH–water (40:40:20).

Table 1
Comparison of capacity factors (k') of molecular species of egg PC and egg PE (derived from egg PC) on different reversed-phase columns[a] [mobile phase: acetonitrile–MeOH–water (49:49:2); UV 208 nm]

Sample	Capacity factor, k'					
	Molecular species[b]					
	1	2	3	4	5	6
(A) PC						
ODPVA	1.10	1.29	1.54	1.85	2.07	2.36
ODS-1	11.7	13.6	15.0	18.5	21.2	24.4
ODS-2	12.2	14.7	17.0	23.3	25.7	27.4
(B) PE						
ODPVA	1.35	1.54	1.76	2.17	2.41	2.76
ODS-1	11.0	12.9	14.9	20.3	22.0	23.5
ODS-2	13.1	15.7	18.5	25.2	28.3	29.5

[a] ODPVA=Asahipak ODP, ODS-1=YMC ODS-A, ODS-2=Prodigy ODS-2, PC=phosphatidylcholine, PE=phosphatidylethanolamine. Fatty acid designations: 16:0=palmitic acid, 18:0=stearic acid, 18:1=oleic acid, 18:2=linoleic acid, 20:4=arachidonic acid, 22:6=docosahexaenoic acid.
[b] Component identification: (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, (6) 18:0–18:2.

column through a Rheodyne (Cotati, CA, USA) Model 7125 injector housed with a 10-μl loop. The lower limit for injection was 100 ng of lipid. Three replicate injections were made for each sample to obtain average retention times (t_R) of PL components. From the t_R values, capacity factors ($k'=t_R/t_o-1$) were determined for each component with

Table 2
Capacity factors (k') of molecular species of PE derivatives (derived from egg PC) on ODPVA [mobile phase: acetonitrile–MeOH–water (47.5:47.5:5); UV 208 nm]

Sample	Capacity factor, k'					
	Molecular species					
	1	2	3	4	5	6
PE	2.27	2.56	2.90	3.68	4.12	4.61
MMPE	2.37	2.76	3.15	3.85	4.41	5.00
DMPE	2.56	2.90	3.29	4.12	4.66	5.20
PC	2.17	2.51	2.85	3.59	4.02	4.51
CAPE	2.22	2.61	3.00	3.68	4.23	4.67
DAPE	2.56	3.01	3.49	4.07	4.76	5.44

For component identification and abbreviations for PC and PE, see footnote to Table 1. MMPE=N-monomethyl-PE, DMPE=N-dimethyl-PE, CAPE=N-caproylamine-PE, DAPE=N-dodecanylamine-PE.

reference to the retention time (t_o) of an unretained solute (uracil or sodium nitrate).

Molecular species of investigated PLs were characterized by HPLC peak matching with those of synthetic standards or by fatty acid analyses [16]. Collected samples of individual HPLC peak components were analyzed as their methyl esters using published capillary gas chromatographic methods [1,16].

3. Results and discussion

As depicted in Fig. 1, three structure types of nitrogenous PLs were investigated. Compounds in type (A) include PC, PE, N-monomethyl-PE (MMPE) and N-dimethyl-PE (DMPE). Compounds in type (B) include N-caproylamine-PE (CAPE) and N-dodecanoylamine-PE (DAPE). Both type (A) and type (B) PLs are glycerophospholipids having glycerol backbone structures. Type (C) compounds are sphingophospholipids with sphingosine backbone structures. Samples of sphingomyelin (SP) derived from animal sources were chosen for study. Each of these PL compounds under consideration contained an internally neutralized charge in the molecule. The basic and ionic properties of these substances usually cause undesirable elution characteristics on ODS with no encapping due to interactions with residual silanol. As logical alternatives, six reversed-phase

Table 3
Influence of mobile phase ammonium hydroxide concentrations on capacity factors (k') of the four major molecular species of egg PC and egg PE (derived from egg PC) on ODPVA (ELSD)

Mobile phase acetonitrile–MeOH–water	Capacity factor, k'			
(x:y:z)	Molecular species			
% NH_4OH	16:0–18:2	16:0–18:1	18:0–18:2	18:0–18:1
(I) x:y:z =47.5:47.5:5				
(A) 0.00%				
PC	2.85	4.02	4.51	6.81
PE	2.90	4.12	4.61	7.01
(B) 0.05%				
PC	2.82	3.91	4.49	6.38
PE	0.90	1.59	1.80	2.46
(C) 0.15%				
PC	2.48	3.40	3.93	5.56
PE	0.15	0.52	0.75	1.24
(D) 0.25%				
PC	1.32	1.71	1.98	2.63
PE	0.03	0.27	0.45	0.70
(II) x:y:z=45:45:10				
(E) 0 00%				
PC	10.4	15.7	18.5	29.7
PE	9.07	14.4	16.0	25.7
(F) 1.00%				
PC	8.54	12.8	15.0	24.1
PE	0.50	1.00	1.73	2.78
(G) 3.00%				
PC	7.29	11.0	12.7	20.5
PE	0.33	0.76	1.34	2.56
(H) 5.00%				
PC	6.78	10.2	11.8	18.9
PE	0.27	0.66	1.25	2.29

HPLC columns including four polymer-based phases and two deactivated ODS phases were initially assessed for the separation of PL molecular species. Among the four polymer columns (see Section 2 for detailed specifications) tested, HPLC with a ODPVA column yielded the most satisfactory separation of the lipid species, as demonstrated in Fig. 2. Under identical mobile phase conditions, experiments with the three other polymer columns [(A), (B) and (D) in Fig. 2] produced similar k' values as those with ODPVA (C), but separations were much less efficient than those obtained with the latter column. The results indicate that silanols are not the only factor determining the performance.

HPLC of nitrogenous PLs on a non-endcapped ODS column embedded with active residual silanol and metals has been reported to produce ill-defined peaks with little component resolution [1]. In this study, we used deactivated, endcapped and/or polymeric ODS columns of low metal content (e.g., YMC ODS-A and Prodigy ODS-2) and obtained reasonable separations of PL molecular species (not shown here). However, analyses of the PLs on these ODS phases invariably required larger amounts of sample materials and produced broader, more strongly retained analyte peaks than the polymer-based ODPVA phase. As shown in Table 1, the k' values of molecular species of egg PC and egg PE (derived from egg PC) resolved on ODPVA were remarkably reduced when compared with those obtained with either of two ODS columns used. The retention data for all corresponding species of PC and PE on ODPVA were parallel to those found in HPLC with the endcapped Prodigy ODS-2 phase. Thus, in both column systems, PC species had lower k' values than the PE counterparts: k'-PC<k'-PE (ODPVA vs. ODS-2, Table 1). On the other hand, in experiments with the endcapped YMC column, the relation between the k' values of corresponding PC- and PE-species were found to be rather erratic (ODPVA vs. ODS-1, Table 1).

Inspection of the HPLC data for PE derivatives in Table 2 revealed that the retention of N-methyl analogues or N-acylamine homologues on ODPVA became progressively stronger (higher k' value) with increasing number of carbon atoms: k' (DMPE)>k' (MMPE)>k' (PE) for PL species in the N-methyl series; k' (DAPE)>k' (CAPE) for the components in the N-acylamine series. Surprisingly, the N-trimethyl analogue, PC, exhibited the weakest retention characteristics (lowest k' value) despite its possession of the largest number of N-methyl groups in the molecule. This apparently anomalous behavior of PC on ODPVA was contradictory to that observed in reversed-phase ion-pair HPLC with an ODS phase [17]. The augmented retention of other N-methyl

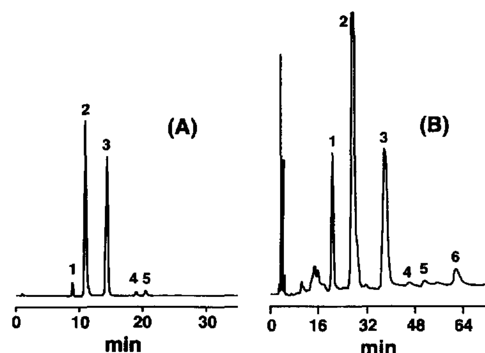

Fig. 3. Reversed-phase HPLC separations of soybean PC on ODPVA. Detection: (A) ELSD, (B) UV. Mobile phases: (A) acetonitrile–MeOH–water (47.5:47.5:5); (B) acetonitrile–MeOH–water (45:45:10). Peak identification: (1) 18:2–18:3, (2) 18:2–18:2, (3) 16:0– 18:2, (4) 16:0–18:1, (5) 18:0–18:2, (6) 18:0–18:1.

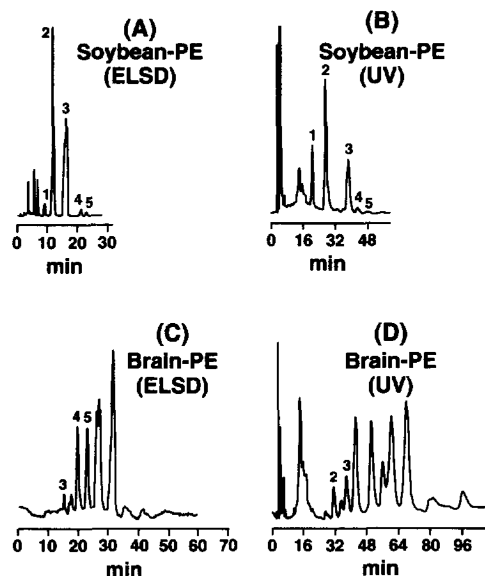

Fig. 4. Reversed-phase HPLC separations of soybean PE and brain PE on ODPVA. Detectors: (A,C) ELSD, (B,D) UV. Mobile phases: (A,C) acetonitrile–MeOH–water (47.5:47.5:5); (B,D) ACN– MeOH–water (45:45:10). For peak identification, see Fig. 3.

analogues might be attributed to specific interactions between the hydroxy moieties of ODPVA and the N–H bonding of the compounds in the series excluding PC. In the absence of such interactions, the k' values of molecular species in the PE derivatives would decrease in the following order: k' (PC)>k' (DMPE)>k' (MMPE)>k' (PE).

The retention data in Table 3 demonstrates a decreasing trend of k' values of the four major molecular species of egg PC and PE on ODPVA with increasing concentrations of ammonium hydroxide in mobile phases. For PC species, the ammonium hydroxide concentration effects on their k' values were much less profound than those for the corresponding PE species. The observed distinct difference in the HPLC behavior of PC and PE species in basic eluents appeared to stem from the structural difference in their nitrogen moieties. In other words, the retention of quaternary ammonium ions (PC) would not be affected as much as that of amines (PE) which underwent a proton dissociation equilibrium with the neutral form by the addition of a base. This would leave an ion instead of an intramolecular ion pair. It was also found that few changes in separation patterns or the column selectivity for the neutrally-charged PL species occurred by the modification of mobile phase basicity.

Comparisons of HPLC–UV and HPLC–ELSD chromatograms (Fig. 3 and Fig. 4) of selected PL samples from plant and animal origins showed that, with the exception of some differences in peak intensities for corresponding peaks, peak patterns of component separations observed in both detection systems were similar. The UV detection technique often led to the appearance of extra UV absorbing peaks that were absent in HPLC–ELSD (Fig. 3A vs. Fig. 3B; Fig. 4A vs. Fig. 4B; Fig. 4C vs. Fig. 4D). Furthermore, because of variations in UV absorbance of individual PL species, the peak intensities were not reflective of the actual amounts of analyte components in a sample. On the other hand, the HPLC–ELSD peak signals were proportional to the concentrations of various PL molecular species in assay samples provided that the molecular mass of

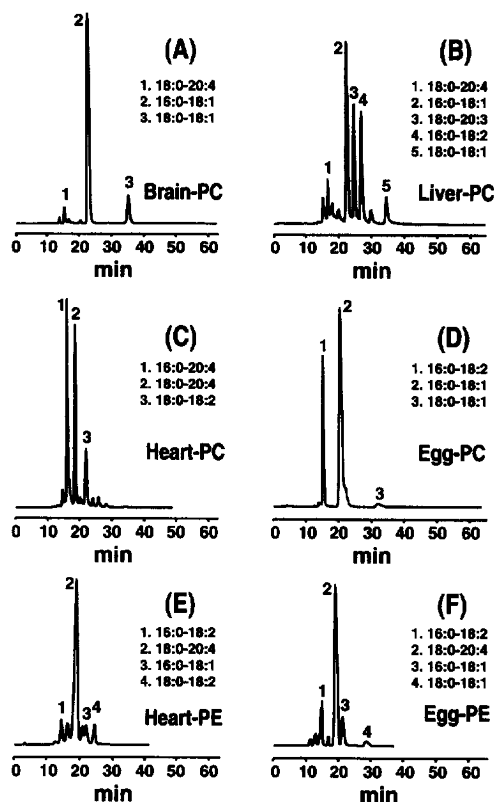

Fig. 5. Reversed-phase HPLC–ELSD separations of PC and PE derived from various animal sources with a ODPVA column and a mobile phase of acetonitrile–MeOH–water (47.5:47.5:5).

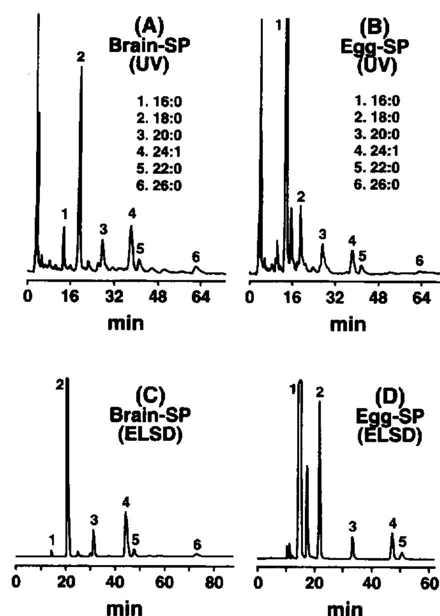

Fig. 6. Comparisons of reversed-phase HPLC–UV and HPLC–ELSD separations of SP on ODPVA with a mobile phase of acetonitrile–MeOH–water (47.5:47.5:5).

the species was not significantly different from each other.

Cursory survey of the chromatograms in Fig. 3 and Fig. 4 indicated that the molecular species derived from animals (e.g., brain PE) were more complex than those from plant sources (e.g., soybean PE). As described in an earlier work [1], the brain PE sample also contained plasmalogen species. In general, the high water content of mobile phases enhanced reversed-phase solvophobic interactions between analyte solutes and the ODPVA phase and thereby improved the separation of the component species (HPLC–UV vs. HPLC–ELSD, Fig. 3 and Fig. 4). HPLC–UV chromatograms often displayed multiple minor components including impurities from animal sample matrices. Overall, UV detection methods seemed to offer greater sensitivity than ELSD methods. The minimum detection limits at a signal to noise ratio of 4:1 were 100 ng and 2 μg for the UV and ELSD methods, respectively.

Additional examples of HPLC–ELSD chromatograms of PC and PE samples derived from animal sources are shown in Fig. 5. Analyses of samples of high PL concentrations indicated that on the expanded chromatograms (not shown), there were numerous minor component peaks emerging alongside the off-scale peaks of the most abundant species. In these cases, peak separations were more complicated than those shown in Fig. 5.

In view of the presence of a common N-trimethylammonium structure in their head groups, the retention behavior of SP on ODPVA should be very similar to PC on the same stationary phase. From the HPLC–UV and HPLC–ELSD profiles in Fig. 6, it is clearly demonstrated that molecular species in both samples of egg SP and brain SP [18,19] were adequately resolved. However, the number and identity of molecular species were different between egg SP and brain SP samples. The most abundant species corresponding to a peak around 14 min in egg SP appeared to be present in the brain SP sample at a much lower level based on the visual estimation of HPLC–ELSD peak heights (Fig. 6B vs. Fig. 6D).

Application of the ODPVA HPLC–ELSD method

Table 4
Reversed-phase HPLC–ELSD analyses of molecular species of PC and PE on ODPVA in plant oil samples [mobile phase: acetonitrile–MeOH–water (47.5:47.5:5)]

Sample[b] number	Composition (%)[a] Molecular species				
	18:2–18:3	18:2–18:2	16:0–18:2	16:0–18:1	18:0–18:2
(A) PC					
Canola oil					
1	TR	5.2	36.9	57.8	ND
2	ND	2.9	40.4	56.7	ND
3	1.2	12.5	44.6	41.8	ND
4	1.2	19.2	44.2	35.4	ND
Soybean oil					
1	2.1	41.9	51.8	2.0	2.2
2	ND	24.2	64.3	10.7	TR
(B) PE					
Canola oil					
1	ND	TR	14.0	84.6	ND
2	2.0	37.1	58.7	1.7	ND
3	TR	2.1	4.4	93.6	ND
4	3.8	21.6	48.4	26.2	ND
Soybean oil					
1	1.2	4.4	73.6	19.5	1.4
2	1.7	19.6	77.2	TR	1.6

[a] Mean values based on three determinations. TR=trace. ND=none detected.
[b] Oil samples represent different varieties of genetically modified oils.

to the analysis of PC and PE in vegetable oil samples derived from various genetically modified oilseeds confirmed the potential utility of the new column technique in practical sample assays. The results are summarized in Table 4. The composition data for PC and PE were obtained from triplicate sample determinations which yielded coefficients of variation ranging from 0.2 to 7.9%. The distribution patterns of PL molecular species varied considerably among different oil samples. From the data in Table 4, it is noteworthy that compositions of some corresponding molecular species were quite different between PC and PE derived from the same sample. Thus, sample 2 of canola oil contained 56.7% 16:0/18:1-PC species, while the corresponding 16:0/18:1-PE species was detected at a much lower level, 1.7% (Table 4). Conversely, levels of the 18:2/18:2 molecular species of PC and PE found in this sample were 2.9 and 37.1%, respectively.

The HPLC method developed in this study was particularly useful in our PL analyses of canola oils because of low concentrations of PC and PE in these oils. The PL contents of canola oils are generally about three fold lower than those of soybean oils. Fig. 7 compares the HPLC–ELSD separations of plant PC samples on two different columns. Routine reversed-phase HPLC analyses of soybean oils for PL molecular species on a commercially prepacked ODS column with a mobile phase of methanol–chloroform–water (30:1:1) have been successful in our laboratory. However, earlier attempts at analyzing PL molecular species in canola oil samples according to the published ODS procedures for soybean oils [13,15] failed to yield satisfactory results owing to insufficient sample materials. The PL analytes were partially adsorbed on the ODS phase. Subsequently, using the ODPVA column as stationary phase, HPLC–ELSD measurements of PL species in canola oils gave acceptable results which were in good agreement with the data obtained with an ODS column (Table 5).

In conclusion, of the three polymer columns evaluated, the best HPLC results were obtained with the ODPVA stationary phase. The ODPVA HPLC methodology provides useful means for rapid, reliable routine analyses of molecular species of neutrally-charged nitrogenous PLs with enhanced detection sensitivity. Since it is not necessary to use

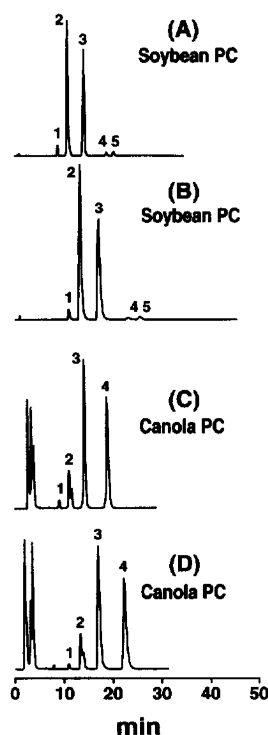

Fig. 7. Comparisons of HPLC–ELSD separations of soybean PC and canola PC on two different columns. Columns: (A,C) ODPVA, (B,D) ODS. Mobile phases: (A,C) acetonitrile–MeOH–water (47.5:47.5:5); (B,D) MeOH–chloroform–water (30:1:1). For peak identification, see Fig. 3.

bufferred mobile phases, detector interfaces are amiable to reversed-phase HPLC analyses employing various hyphenated techniques such as HPLC–ELSD and HPLC–MS. With ELSD, the PL molecular species can be simultaneously separated and quantitated. Normally, baseline separations of molecular species of the title compounds can be achieved by optimization of mobile phase conditions. The reversed-phase ODPVA HPLC methods can be applied to the trace analysis of PC- and PE-molecular species in various sample matrices. HPLC of the PL molecular species on ODPVA provided significantly faster analysis and greater detection sensitivity than assays with deactivated octadecylsilica columns. The solvent systems used in ODPVA HPLC of PLs are environmentally more acceptable than volatile organic solvents employed in HPLC with the ODS phase where chloroform is used as the co-solvent.

Table 5
Reversed-phase HPLC–ELSD analyses of molecular species of PC in canola oils using two different columns [mobile phase: acetonitrile–MeOH–water (47.5:47.5:5)]

Sample[b] number	Composition (%)[a] Molecular species				
	18:2–18:3	18:2–18:2	16:0–18:2	16:0–18:1	18:0–18:2
1					
ODPVA	1.87	39.8	59.3	ND	ND
ODS	5.15	36.9	57.8	ND	ND
2					
ODPVA	2.48	39.8	58.0	ND	ND
ODS	2.85	40.4	56.8	ND	ND
3					
ODPVA	1.68	13.2	43.8	41.2	ND
ODS	1.17	12.5	44.6	41.8	ND
4					
ODPVA	1.34	17.6	47.5	33.6	ND
ODS	1.24	19.2	44.2	35.4	ND

[a] Mean values based on three determinations. TR=trace. ND=none detected.
[b] Oil samples represent different varieties of genetically modified oils.

References

[1] S.L. Abidi, T.L. Mounts, J. Chromatogr. 598 (1992) 209.
[2] A. Cantafora, R. Masella, J. Chromatogr. 593 (1992) 139.
[3] R.L. Glass, J. Agric. Food Chem. 38 (1990) 1684.
[4] N. Sotirhos, C. Thorngren, B. Herslof, J. Chromatogr. 331 (1985) 313.
[5] A. Cantafora, A. Dibiase, D. Alvaro, M. Angelico, M. Marin, A.F. Attili, Clin. Chim. Acta 134 (1983) 281.
[6] B.J. Compton, W.C. Purdy, Anal. Chim. Acta 141 (1982) 405.
[7] G.M. Patton, J.M. Fasulo, S.J. Robins, J. Lipid Res. 23 (1982) 190.
[8] M. Smith, F.B. Jungalwala, J. Lipid Res. 22 (1981) 697.
[9] B.J. Compton, W.C. Purdy, J. Liq. Chromatogr. 3 (1980) 1183.
[10] N.A. Porter, R.A. Wolf, J.R. Nixon, Lipids 14 (1979) 757.
[11] W. Christie, M.L. Hunter, J. Chromatogr. 325 (1985) 473.
[12] S.L. Abidi, T.L. Mounts, Presented at the 20th International Symposium on High Performance Liquid Phase Separations and Related Techniques, 16–21 June, 1996, Book of Abstracts, P-0101, p. 71.
[13] T.L. Mounts, S.L. Abidi, K.A. Rennick, J. Am. Oil Chem. Soc. 69 (1992) 438.
[14] S.L. Abidi, T.L. Mounts, T. Finn, J. Am. Oil Chem. Soc. 73(4) (1996) 535.
[15] T.L. Mounts, S.L. Abidi, K.A. Rennick, J. Am. Oil Chem. Soc. 73(5) (1996) 581.
[16] W.W. Christie, Lipid Analysis, Pergamon Press, New York, NY, 1973, p. 85.
[17] S.L. Abidi, T.L. Mounts, J. Chromatogr. A 741 (1996) 213.
[18] F.B. Jungalwala, V. Hayssen, J.M. Pasquini, R.H. McCluer, J. Lipid Res. 20 (1979) 579.
[19] M. Smith, P. Monchamp, F.B. Jungalwala, J. Lipid Res. 22 (1981) 714.