Analysis of Individual Phospholipids by High-Performance Capillary Electrophoresis

Lisbeth Ingvardsen, Søren Michaelsen and **Hilmer Sørensen***
Chemistry Department, Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark

A method of analysis for determination of phospholipids by micellar electrokinetic capillary chromatography (MECC) has been developed. Sodium cholate (NaCh) was found suitable as the micellar phase, and 1-propanol was important in obtaining an efficient separation of individual phospholipids and as organic solvent required for acceptable solubility of the phospholipids. With equal volatility of water and organic modifier, only minor changes in effects from the modifier occur during analysis. The influence of changes in high-performance capillary electrophoresis–MECC separation conditions were evaluated in terms of migration time, relative migration time (RMT), relative normalized area (RNA), resolution and theoretical plates per meter. The method has several advantages compared to high-performance liquid chromatography: The total time of analysis is less than 25 min, and only small amounts of reagents and sample are required. Relative standard deviations were 0.26–0.48% for RMT, 1.7–2.9% for RNA, and in the linearity test correlation coefficients of 0.999 were obtained. Results from analyses of the phospholipid compositions of soybean and rapeseed lecithins are presented.

KEY WORDS: Capillary electrophoresis, micellar electrokinetic chromatography, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phospholipids.

Phospholipids are ubiquitous in nature, occurring in biological membranes of plants and animals. These compounds give cell membranes structural continuity and influence membrane macromolecular function, and they are involved in membrane signal transduction and in the nervous system. Interest in phospholipids is therefore often related to their biochemical and functional activities, both *in vivo* and *in vitro* (1), and to the structure of the individual compounds in relation to their use in cosmetics, pharmaceutical carriers, pharmacological agents and foods.

Phospholipids, abundant in lecithins, are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS), but lecithins contain several other constituents in minor amounts (1). Commercially important lecithins are those from soybean, rapeseed, egg, cottonseed and other oilseed plants. Sosada *et al.* (2) found that especially rapeseed lecithins from 00-varieties (low in erucic acid and glucosinolates) offer the chance to increase applications in the food industry, cosmetics and pharmaceuticals.

Accurate analysis of lecithins and phospholipids are increasingly important, both for producers and for users. Recently, high-performance liquid chromatography (HPLC) has been developed as a method of analysis for separation and quantitation of phospholipids (3,4). Analysis of derivatized phospholipids, produced by hydrolysis of phospholipids catalyzed by phospholipase C and then derivatized with the chromophor anthroyl chloride, has also been described (5–7). However, these methods are experimentally rather complicated, relatively expensive and time-consuming, and proper identification of individual phospholipids can be difficult. We found an urgent need for cheap, fast, simple and efficient methods of analysis by which individual phospholipids could be determined.

Since the introduction of high-performance capillary electrophoresis (HPCE), based on micellar electrokinetic capillary chromatography (MECC), by Terabe *et al.* in 1984 (8), this technique has been developed into powerful and advantageous methods. This is due to the higher peak capacity, resolution and improved reproducibility compared with HPLC. Our purpose was to develop a method of analysis for phospholipids by use of HPCE and the MECC system. For comparison, we focussed on literature concerning HPCE analysis of compounds with properties similar to the phospholipids. Methods devoted to separation of hydrophobic molecules have been described (9–12), as have methods devoted to determination of negatively charged compounds (13), but the lacking hydrophobicity of these analytes makes the methods inapplicable to phospholipids. With the combined technique of group separation, purification and MECC now developed, a rapid and efficient method of analysis for individual phospholipids can be applied successfully to various vegetable oils and lecithins, as well as to phospholipids used in the industries.

MATERIALS

Chemicals and reagents were of at least analytical reagent grade. 1-Propanol was of HPLC grade; chloroform, methanol, ethanol, 25% ammonia, 98% formic acid, 90% acetic acid, ammonium sulfate, di-sodium hydrogenphosphate, di-sodium tetraborate and sodium cholate (NaCh), all of guaranteed reagent grade, were from Sigma (St. Louis, MO). Phospholipids used for standard solutions were: L-α-PI from bovine liver, L-α-phosphatidyl-L-serine from bovine brain, L-α-PE from soybean and synthetic dioleoyl-L-α-PC purchased from Sigma. Preparations of various phospholipids were isolated from commercial soybean lecithin and rapeseed lecithin (*vide infra*) donated by Aarhus Oliefabrik A/S (Aarhus, Denmark).

METHODS

Isolation of individual phospholipids from commercial lecithin. Lecithin (100 mg) was dissolved in 2 mL chloroform/ethanol (1:1, vol/vol) and centrifuged 10 min at 5000 rpm in a Labofuge 200 (Heraus Sepatech, Osterode, Germany). The supernatant (250 μL) was transferred to a Dowex 1 × 8, 200–400 mesh (AcO^-) (Merck, Darmstadt, Germany) column (5.0 × 0.5 cm i.d.), which initially was washed with 2 mL of chloroform/ethanol (1:1, vol/vol). Two-step elution was performed on a Visiprep Vacuum Manifold (Supelco, Bellefonte, PA). The fractions obtained were an effluent obtained by use of 20 mL chloroform/ethanol (1:1, vol/vol), followed by an eluate obtained by

*To whom correspondence should be addressed at Biochemical Research Group, Chemistry Department, Royal Veterinary and Agricultural University, 40, Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark.

use of 10 mL chloroform/ethanol/formic acid (4.5:4.5:1.0, vol/vol/vol). Separation of phospholipids in the eluates was achieved by preparative thin-layer chromatography (TLC) (DC-alufolien, Kiselgel 60 without fluorescent; Merck) developed in either chloroform/methanol/acetic acid/water (60:30:8.4:3.6, by vol) or chloroform/methanol/25% ammonia (68:28:4, vol/vol/vol). The plates were dried, and strips were developed by spraying to saturation with 5% ammonium sulfate in 50% ethanol and heating to 175°C for 45 min. Phospholipids were eluted from the silica material with the respective developing solution. Isolates were evaporated and redissolved in chloroform.

Preparation of sample solution. High concentrations of phospholipids in chloroform (about 10 mg/mL) were diluted to appropriate concentrations with 5% (116 mM) NaCh in 50% 1-propanol. At least eight times dilution should be used to obtain a homogeneous sample solution.

HPCE analysis. HPCE was performed with an ABI Model 270A capillary electrophoresis system (Applied Biosystems, Foster City, CA) and a 760 mm × 0.05 mm i.d. fused-silica capillary tube. Detection was carried out at a position 530 mm from the injection end of the capillary by on-column measurements of ultraviolet (UV) absorption at 200 nm. For data processing, a Shimadzu (Kyoto, Japan) Chromatopac C-R3A was used. The separations were performed at 50°C and 30 kV.

The separation buffer was a solution of 75 mM NaCh, 10 mM di-sodium hydrogenphosphate, 6 mM di-sodium tetraborate and 30% 1-propanol adjusted to pH 8.5 with 1 M HCl. Prior to use, the buffer was filtered through a 0.20-μm membrane filter.

All samples were dissolved as described (*vide supra*) and introduced from the positive end of the capillary by vacuum for 3 s. When running several successive samples, the capillary was washed with 1.0 M NaOH for 4 min, followed by buffer for 10 min after each analysis. The same was done when the buffer composition was altered.

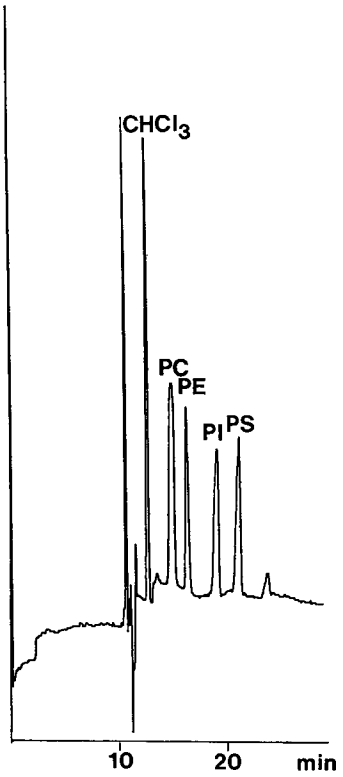

FIG. 1. Electropherogram of phospholipids separated by the micellar electrokinetic capillary chromatography method. Separation conditions: Buffer composition of 75 mM NaCh, 10 mM di-sodium hydrogenphosphate, 6 mM di-sodium tetraborate and 30% 1-propanol, pH adjusted to 8.5 with 1 M hydrogen chloride; temperature 50°C; voltage 30 kV; total length of capillary 760 mm and detection 530 mm from injection end; ultraviolet detection at 200 nm. Vacuum injection for 3 s. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

RESULTS AND DISCUSSION

HPCE-MECC could indeed overcome the analytical difficulties encountered in the determination of individual phospholipids. The influence of various solvents was studied because phospholipids have an expected tendency to renewed membrane/bilayer/micelle formation in the aqueous environment required for MECC. Promising results were obtained when NaCh was used as the micellar phase (14). The explanation for this is probably related to the structure of the micelles because NaCh, at concentrations above the critical micelle concentration (CMC; 5–10 mM), has been suggested to form rodlike or cylindrical micelles, with the hydrophobic part situated on the surface and the hydrophilic part turned inward (12,15,16). These inverse micelles favor hydrophobic interaction with the phospholipids, which are either neutral or negatively charged. Separation can thus occur as a consequence of different partitioning coefficients for the analytes between the micellar phase and the aqueous phase.

Separation of the phospholipids in a standard mixture of PC, PE, PI and PS was efficiently achieved (Fig. 1). Identification of the peaks, corresponding to the individual compounds, was based on use of authentic compounds and defined by relative migration time (RMT) values. Calculation of RMT was performed with chloroform as the reference compound, which also acts as a marker for the electroendoosmotic flow (EOF). Phospholipid standard solutions were obtained by mixing the single phospholipids and diluting to appropriate concentration with 5% NaCh in 50% 1-propanol. When electropherograms of standard solutions were recorded after a few days, PC could appear as a double peak, even if stored at 0–4°C. However, this phenomenon was not observed with phospholipid samples preparatively isolated from rapeseed and soybean lecithins.

Development of the method now described has comprised systematic investigations based on different separation conditions. They covered changes of temperature, voltage and composition of the separation buffer, including pH and concentrations of NaCh, 1-propanol and phosphate/borate (electrolyte). Influences from changes of those conditions on migration time (MT), RMT, normalized peak area (NA), resolution (R_s) and theoretical plates per meter (N) were calculated as described elsewhere (17).

It was found that pH of the buffer had a great influence on MT of the solutes (Fig. 2). A considerable decrease in MT for all of the compounds was seen by changing the pH value from 7.5 to 8.5, which affected the hydrophobic

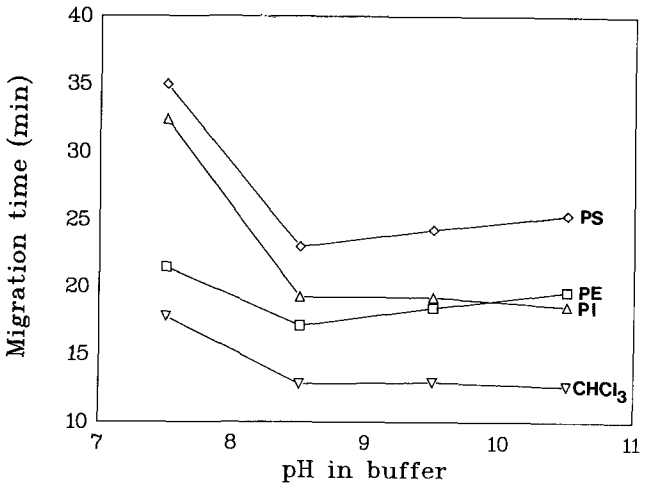

FIG. 2. Relationship between pH and migration times of phospholipids. Abbreviations and separation conditions other than pH as in Figure 1.

properties of the micelles on account of the cholate's degree of ionization (pK_a 6.4). At pH values above 8.5, the changes in MT values were not as remarkable, but the MTs for PE and PI approached each other. This resulted even in a shift of migration order for PE and PI at pH 10.5. These changes result from an altered net charge of the analytes and hence their migration through the capillary. Especially, the net charge for PE and PS will be changed at high pH values, due to approaching the pK_a value for the amino group. The net charge of the analytes has a direct influence on MT, but interaction between analytes and micelles is another factor responsible for the changed MT. RMT changes in a nonsystematic way, and pH affects the NA for PE, which is reduced at pH 8.5 from that at pH 7.5. The results presented in Figure 2 explain also the effects on R_s values, which decreased for PE–PI and increased for PI–PS with increasing pH. The separation distance between PE and PI thus became smaller when pH was above 8.5. In addition, the highest N values were obtained at pH 8.5, although N was only slightly changed for PI and PS, whereas a considerable increase in N for PE was seen by changing the pH from 7.5 to 8.5. This was followed by a lesser decrease at higher pH values. By increasing pH from 7.5 to 8.5, about 30% reduction in the time of analysis was obtained. Based on these observations, pH 8.5 was chosen as the preferred value.

Increasing the cholate concentration from 25 mM to 100 mM resulted in a hyperbolic increase in MT for all of the analytes. RMT changed in the same way, although the increase was less pronounced (Fig. 3A). MT increased as a result of a higher NaCh concentration, and therefore it followed the increased phase ratio, *i.e.*, the ratio of the volume of the micellar phase to that of the aqueous phase. Furthermore, the increase in MT of chloroform demonstrates the influence of the NaCh concentration on EOF.

Increasing the cholate concentration reduced NA, except for PI at 50 mM and PS at 75 mM, which showed increases (Fig. 3B). The decrease in NA with increased concentration of NaCh could be caused by altered micellar structure and increased interaction between the micelles and the phospholipids. Analytes included in the micelles will cause different absorbance of UV radiation compared to the free analytes, resulting in a changed peak area for the same analyte concentration. The resulting change toward an increasing amount of phospholipid bound to or present in the micellar phase with increasing concentration of NaCh explains the effects observed for both MT and NA. The results are thus in agreement with the expected tendency of phospholipids to give renewed membrane/bilayer/micelle formation in the aqueous environment required for MECC. R_s values increased with increasing cholate concentration, and N changed only for PE, where an increase was seen by changing from 25 to 50 mM cholate (thereafter it was decreasing). However, to obtain an acceptable distance between the peak caused by chloroform and the peaks from the phospholipids, further investigations were performed at 75 mM NaCh.

The modifier 1-propanol had great influence on the separation of phospholipids. Less than 20% 1-propanol gave insufficient separation of PE and PC. Therefore, only results for 1-propanol concentrations above 20% were of interest. MT increased more than 50% by increasing the 1-propanol concentration from 30 to 40%, whereas RMT values of the analytes and chloroform were nearly unaffected in this concentration area (Fig. 3C). NA decreased considerably with increasing content of 1-propanol in the buffer. The effects on MT and NA caused by 1-propanol indicate an increased interaction between the analytes and the micelles with increased 1-propanol concentration, similar to the effects of increasing NaCh concentration (*vide supra*). Both R_s and N were improved at higher concentration of 1-propanol. The results obtained for N are illustrated in Figure 3D. Presence of the organic modifier in the buffer reduced EOF, due to a reduction in the zeta potential (18,19), and increased MT for the analytes. However, the organic modifier was expected to lower the capacity factor, k′, for the solutes (20), resulting in reduced interaction between the micelles and the phospholipids, but this is not in agreement with the effects observed for NA and MT when the 1-propanol concentration was increased (*vide supra*). Based on these experiences, we concluded that quantitative determination of phospholipids requires use of an internal standard and a well-defined system. To avoid long analysis times, 30% 1-propanol was chosen for further investigations.

Looking at the influence of electrolyte concentration (from 8 to 32 mM phosphate plus borate in a concentration ratio of 3:5), MT and RMT values remained relatively constant. A decrease in NA was seen with increasing concentration of electrolyte, but R_s for PI–PS and N for all analytes were improved with high electrolyte concentration. Because higher electrolyte concentrations in the buffer reduce EOF (21), MT values of the analytes are expected to decrease. The fact that the observed MT was unaffected may be explained either by a combination of reduced EOF and changes in the micellar phase caused by higher ionic strength or by the high concentration of 1-propanol disturbing the electrical double layer so much that it dominates the changes in electrolyte concentration. Increasing the electrolyte concentration was limited because a trial with 80 mM electrolyte (50 mM phosphate + 30 mM borate) resulted in failed analysis due to

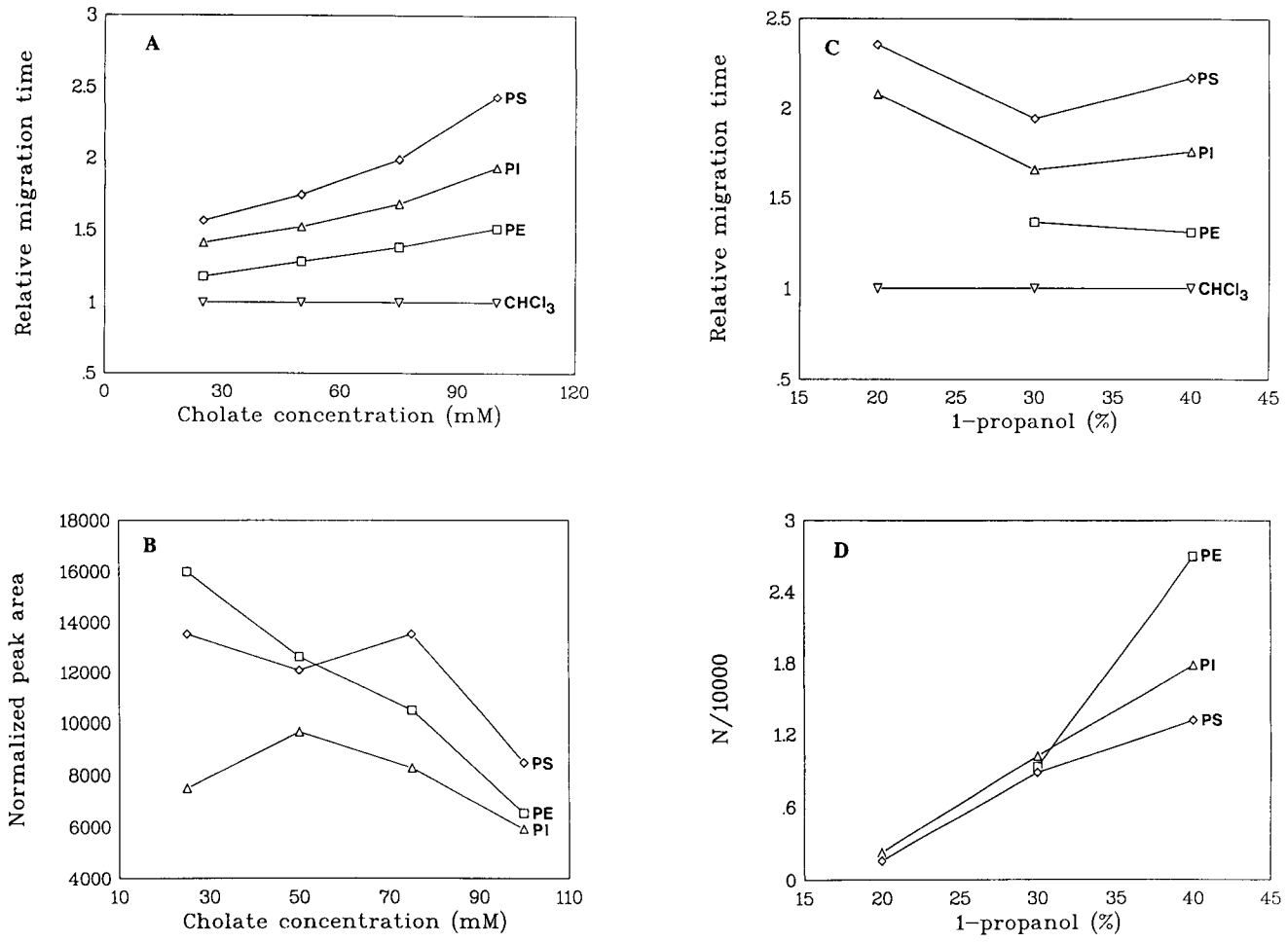

FIG. 3. Effects of varying cholate (NaCh) concentration on relative migration time (RMT) for the phospholipids are shown in A, and NaCh effects on normalized peak areas are shown in B. Relationship between 1-propanol concentration and RMT is shown in C, and the relationship between 1-propanol concentration and number of theoretical plates per meter (N) is shown in D. Abbreviations for the names of phospholipids and separation conditions other than the variable, as in Figure 1.

electrical disturbances caused by the high current. However, high electrolyte concentration in the buffer compared to the electrolyte concentration in the sample, improved the stacking effect, which resulted in higher values of R_s and N. The selected electrolyte concentration for the method is a compromise between positive and negative effects caused by the electrolytes.

Increasing the temperature from 40 to 60°C gave reduced MT and nearly unaffected RMT. NA decreased with temperatures from 40 to 50°C but increased at higher temperatures. R_s changed in a nonsystematic way, whereas N increased slightly for all compounds with increasing temperature. The higher velocities of the analytes with higher temperatures are a result of reduced buffer viscosity and altered association of the analytes to the micellar phase. In regard to the higher risk of electrical disturbances, too-high temperatures should be avoided, and 50°C was chosen.

The last parameter investigated was the applied voltage. Increase of the voltage from 20 to 30 kV reduced MT without effect on RMT. With increasing voltage, NA increased for PE between 25 and 30 kV, and R_s was reduced remarkably for PI-PS and improved a little for PE-PI. N was nearly unaffected, except for PE, where a considerable decrease was observed when the voltage was increased. The reduction in MT with increasing voltage agrees with the inverse relationship between applied voltage and analysis time (20). Due to the advantages of shorter analysis times, we selected 30 kV. Under the chosen separation conditions, the number of theoretical plates per meter of capillary were 28.500, 16.400 and 25.300 for PE, PI and PS, respectively. R_s values for PC-PE, PE-PI and PI-PS were calculated to be 1.98, 4.04 and 2.74, respectively.

As can be seen, it is possible to manipulate migration time significantly by changing the separation parameters discussed in this work. A broader elution range can be obtained, which can be of interest when analyzing solutions containing many different phospholipids.

For the standard solution of phospholipids containing PC, PE, PI and PS, repeatabilities of MT, RMT (using chloroform as reference compound), NA and RNA (using

TABLE 1

Relative Standard Deviation of Migration Times (MT), Relative Migration Times (RMT), Normalized Peak Areas (NA) and Relative Normalized Peak Areas (RNA) for Phospholipids[a]

Phospholipid	Relative standard deviation (%)				
	MT	RMT	NA	RNA[b]	RNA[c]
PC	1.46	0.26	6.49	2.86	1.74
PE	1.12	0.31	5.64	1.74	—
PI	1.66	0.47	5.31	2.53	2.35
PS	1.56	0.48	4.36	—	1.75

[a]Separation conditions as in Figure 1. Phospholipid abbreviations as in Materials. For all calculations n = 7. Abbreviations: PC, phosphatidylcholine; PI, phosphatidylinositol.
[b]Relative to phosphatidylserine (PS).
[c]Relative to phosphatidylethanolamine (PE).

either PS or PE as reference compound) were evaluated, and the results are shown in Table 1. The experiments were done by changing the buffer at the inlet side after each analysis. Uncertainty caused by evaporation from sample vials during the test (17) was minimized by using anti-evaporation septa on the vials. Under the conditions mentioned, the instrument performed well with respect to repeatability. When RMT, NA and RNA values were used, compared to MT and uncorrected peak areas, the repeatabilities, expressed as R.S.D. values, were reduced considerably. For quantitation of the analytes, the results indicate the necessity of an internal standard, which ideally should have the same properties as the phospholipids.

Linearity was determined as the correlation between decreasing concentrations of PC, PE, PI and PS and their corresponding NA values. The results are shown in Table 2. Correlation coefficients for the different phospholipids were between 0.9984 and 0.9998. The tests were made without an internal standard, which probably would have increased the correlation coefficients (22). The linear increase in NA with increasing concentrations of injected phospholipids shows that the method now developed may be used to quantitate phospholipids. However, an internal standard and response factors, determined from the results obtained when testing the linearity, are recommended.

Phospholipids in commercial rapeseed lecithin were separated by anion-exchange chromatography as described in the experimental part. The fractions obtained, corresponding to the effluent (A*) and the eluate (B*), were then analyzed by the developed HPCE method (Fig. 4). The electropherogram of A* (Fig. 4 A*) was quantitatively dominated by several peaks with RMT corresponding to PC compounds (PC derivatives) and a quantitatively dominating group of peaks with RMT greater than found for PS in the standard mixture (Fig. 1). The electropherogram of B* (Fig. 4 B*) has peaks of appreciable sizes with RMT values corresponding to PC and PI (Fig. 1), a minor peak with RMT corresponding to PS and quantitatively dominating in this fraction are compounds with RMT greater than found for PS. Lecithin from rapeseed seems thus to be a mixture of several types of phospholipids. This is in agreement with data published in the literature (23), although great variations are found between these data and those reported by other groups (24). Identification of the structure of the individual compounds requires determination of the fatty acid composition of individual phospholipids, PC, PE, PI, PS and other types.

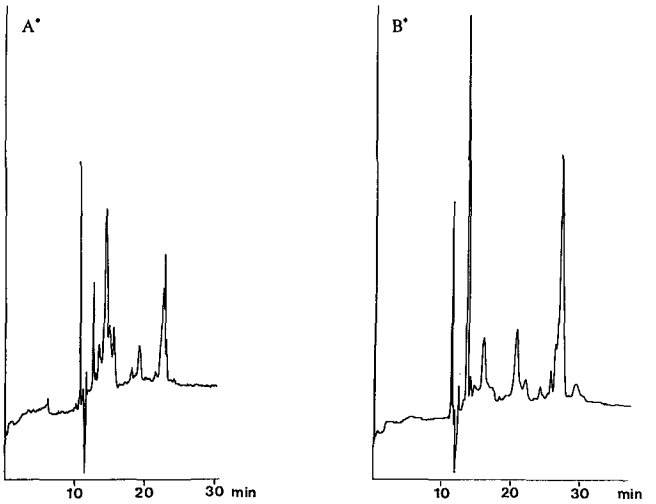

FIG. 4. Electropherograms of phospholipids isolated from rapeseed lecithin. A* is the effluent and B* is the eluate from anion-exchange chromatography. High-performance capillary electrophoresis separation conditions as in Figure 1, and the first peak after the solvent peak in both electropherograms represents $CHCl_3$ as in Figure 1.

Phospholipids in commercial soybean lecithin were separated by anion-exchange chromatography as used for rapeseed lecithin, and further purified by preparative TLC (*vide supra*). Four fractions thus purified had R_f values, for TLC in both of the described mobile phases, corresponding to: a) $R_f < PC_{(ref.)}$; b) $R_f \approx PC_{(ref.)}$; c) $R_f \approx PI_{(ref.)}$; d) $R_f \approx$ diphosphatidylglycerol ($DPG_{(ref.)}$). These four fractions of soybean lecithin were analyzed by HPCE under two different combinations of MECC conditions (Fig. 5). Figure 5 shows that the different MECC conditions give different information. Higher R_s is obtained by the reduced kV and NaCh concentration. Different types of phospholipids are revealed in the soybean lecithins fractions, within the groups corresponding to PC, PE, PI, PS and DPG, respectively, as was the case for rapeseed lecithins. The method of phospholipid analysis now developed as a HPCE-MECC technique gives promising possibilities for determining individual phospholipids that occur in various vegetable oils.

TABLE 2

Linearity Test[a]

Phospholipid	Concentration range (%)	r^2
PC	5-100	0.9996
PE	5-100	0.9988
PI	5-100	0.9998
PS	5-100	0.9984

[a]Correlation coefficients (r^2) from linear regression analyses by least squares method of normalized areas (NA) for various concentrations of phospholipids. Conditions and abbreviations as in Table 1. For all calculations, concentrations of 5, 10, 20, 40, 50, 80 and 100% were investigated.

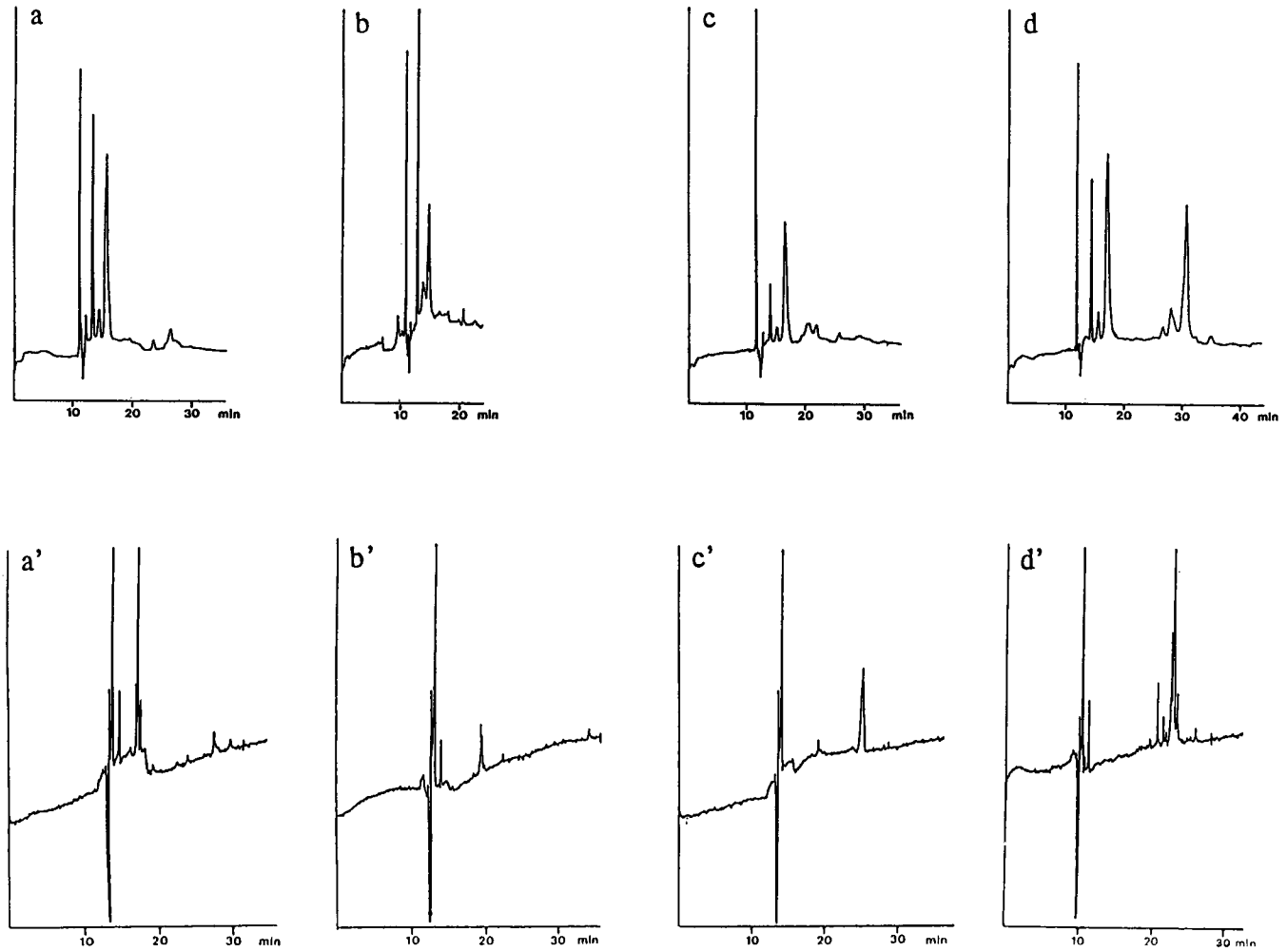

FIG. 5. Electropherograms of phospholipids isolated from soyben lecithin. Four fractions obtained by preparative thin-layer chromatography, as described in the text, were analyzed by conditions as in Figure 4 (a, b, c, d) and by micellar electrokinetic capillary chromatography conditions changed to 4 mM electrolyte, 50 mM NaCh, 25 kV and 50°C (a', b', c', d').

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from the Danish Agricultural and Veterinary Research Council, from the Danish Natural Research Council and from the Danish Agricultural Ministry.

REFERENCES

1. Scholfield, C.R., B.F. Szuhaj and G.R. Lind, *Lecithins*, pp. 1–20, American Oil Chemists' Society, 1985.
2. Sosada, M., B. Pasker and K. Kot, *Fat. Sci. Technol. 94*:233 (1992).
3. Breton, L., B. Serkiz, J.P. Volland and J. Lepagnol, *J. Chromatogr. 497*:243 (1989).
4. Redden, P.R., and Y.-S. Huang, *Ibid. 567*:21 (1991).
5. Kito, M., H. Takamura, H. Narita and R. Urade, *J. Biochem. 98*:327 (1985).
6. Ramesha, C.S., W. Pickett and D.V.K. Murthy, *J. Chromatogr. 491*:37 (1989).
7. Takamura, H., and M. Kito, *J. Biochem. 109*:436 (1991).
8. Terabe, S., K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem. 56*:111 (1984).
9. Nishi, H., T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr. 513*:279 (1990).
10. Nishi, H., T. Fukuyama, M. Matsuo and S. Terabe, *Ibid. 498*:313 (1990).
11. Nishi, H., and S. Terabe, *Electrophoresis 11*:691 (1990).
12. Cole, R.O., M.J. Sepaniak, W.L. Hinze, J. Gorse and K. Oldiges, *J. Chromatogr. 557*:113 (1991).
13. Bjergegaard, C., S. Michaelsen, P. Møller and H. Sørensen, *Ibid. 608*:403 (1992).
14. Bjergegaard, C., L. Ingvardsen and H. Sørensen, *Ibid. 653*:99 (1993).
15. Kawamura, H., M. Manabe, T. Narikiyo, H. Igimi, Y. Murata, G. Sugihura and M. Tanaka, *J. Solut. Chem. 16*:433 (1987).
16. Campanelli, A.R., S.C.D. Sanctis, E. Chiessi, M. D'Alagni, E. Giglio and L. Scaramuzza, *J. Phys. Chem. 93*:1536 (1989).
17. Michaelsen, S., R. Møller and H. Sørensen, *J. Chromatogr. 608*:363 (1992).
18. Balchunas, A.T., D.F. Swaile, A.C. Powell and M.J. Sepaniak, *Sep. Sc. and Technol. 23*:1891 (1988).
19. Schwer, C., and E. Kenndler, *Anal. Chem. 63*:1801 (1991).
20. Balchunas, A.T., and M.J. Sepaniak, *Ibid. 60*:617 (1988).
21. Tsuda, T., K. Nomura and G. Nakagawa, *J. Chromatogr. 248*:241 (1982).
22. Otsuka, K., S. Terabe and T. Ando, *Ibid. 396*:350 (1987).
23. Sosulski, F., R. Zadernowski, J. Little and B. McDonald, *J. Am. Oil Chem. Soc. 58*:561 (1981).
24. Larsson, I., L. Rydhag and I. Wilton, in *Proceedings of the 5th International Rapeseed Conference*, Malmö, Sweden, Vol. 2, 187, 1978.

[Received July 19, 1993; accepted November 16, 1993]