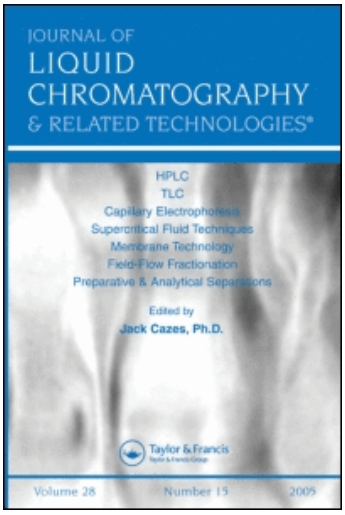

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COMPARISON OF MOBILE PHASES FOR SEPARATION OF PHOSPHOLIPIDS BY ONE-DIMENSIONAL TLC ON PREADSORBENT HIGH PERFORMANCE SILICA GEL PLATES

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ABSTRACT

Eight solvent systems reported in the literature for the one-dimensional TLC separation of phospholipids were compared under identical conditions using high performance preadsorbent silica gel plates. The best overall separation of phospholipid standards was obtained by a single development with chloroform-methanol-water (65:25:4), and three other systems containing chloroform also gave good separations. R_f data are tabulated for these four systems, and the phospholipids extracted from the digestive gland-gonad complex of Biomphalaria glabrata snails are identified.

INTRODUCTION

In an earlier study (1), twenty-four solvent systems were compared for their ability to separate neutral and polar

lipid classes on high performance preadsorbent silica gel plates. A large number of other mobile phases have been reported in the literature specifically for the resolution of phospholipids, and we report in this paper the evaluation of eight of the most promising systems on HP preadsorbent silica gel plates from a single manufacturer under identical conditions, involving single or multiple one-dimensional development of constant amounts of 16 phospholipid standards and extract of the digestive gland-gonad (DGG) complex of Biomphalaria glabrata snails and detection with cupric sulfate-phosphoric acid reagent. R_f values are tabulated for the four best systems, and the phospholipids present in the snail extract are identified.

EXPERIMENTAL

Whatman 20 x 10 cm LHP-KDF silica gel plates were cleaned by development with chloroform-methanol (1:1) and spotted with 5 ul of various phospholipid standards and lipid extract of the DGG from lettuce-fed B. glabrata snails as previously described (1). The standards were purchased from Matreya, Inc. (Pleasant Gap, PA) and are identified in the legend of Figure 1. The individual standards, cerebrosides, and sulfatides were prepared as 1 ug/ul solutions in chloroform-methanol (2:1). The sphingolipid mixture contained 0.33 ug/ul each of cerebrosides, sulfatides, and sphingomyelin. The polar lipid mixture contained 0.25 ug/ul each of cholesterol, phosphatidylethanolamine, lecithin

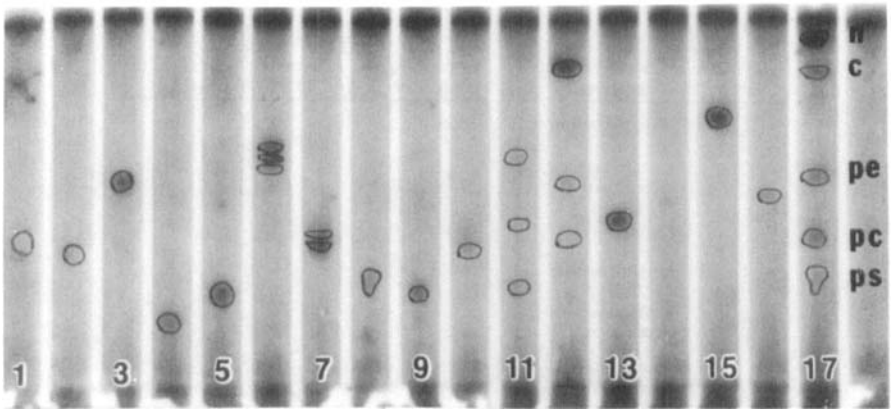

FIGURE 1. Photograph of chromatograms developed with solvent system 1: Lane 1, phosphatidylcholine (pc); 2, phosphatidic acid; 3, phosphatidylethanolamine (pe); 4, lysolecithin; 5, sphingomyelin; 6, cerebrosides; 7, sulfatides; 8, phosphatidylserine (ps); 9, phosphatidylinositol; 10, phosphatidylglycerol; 11, sphingolipid mixture; 12, polar lipid mixture; 13, cardiolipin; 14, lysophosphatidylethanolamine (not detected); 15, monogalactosyldiglyceride; 16, digalactosyldiglyceride; 17, DGG extract of B. glabrata snails. Additional compound abbreviations: cholesterol (c), neutral lipids (n). Zones have been circled to enhance their visibility in the photograph. See text for chromatographic details.

(phosphatidylcholine), and lysolecithin (lysophosphatidylcholine).

The solvent systems tested and development distances are listed in Table 1. Solvents were prepared and ascending developments performed as previously described (1). All of the systems were single development except numbers 5 and 6, in which double development with intermediate drying was carried out. Phospholipids were detected by spraying with cupric sulfate-phosphoric acid solution and heating in a gravity convection oven at 160°C for 10 minutes. The reagent

TABLE 1

Solvent Systems

Solvent number	System components	Volume ratio of components	Solvent front (cm from origin)	Ref
1	Chloroform-methanol-H_2O	65:25:4	7.5	2
2	Chloroform-hexane-methanol-acetic acid-H_2O	12:7:4:3:0.3	7.5	3
3	Chloroform-methanol-H_2O	55:16:25	7.5	4
4	Chloroform-methanol-H_2O	31:18:1	7.5	5
5	Chloroform-methanol-acetic acid-H_2O	60:35:2:1	7.5	
	Chloroform-methanol-acetic acid-H_2O	50:30:8:4	3.0	6
6	Methyl acetate-isopropanol-chloroform-methanol-0.5% KCl in H_2O	25:25:25:10:9	4.5	7
	Hexane-ether-acetic acid	80:20:2	7.5	
7	Chloroform-methanol-acetic aicd-H_2O	60:14:13:2.4	7.5	8
8	Chloroform-methanol acetic acid-H_2O	75:45:3:1	7.5	9

was prepared by diluting 100 g of $CuSO_4$ plus 80 ml of H_3PO_4 to 1 liter with water and stirring magnetically for 1 hour.

RESULTS AND DISCUSSION

Table 1 contains the mobile phases tested and development distances for each from the origin (the silica gel/preadsorbent junction). Cupric acetate-acetic acid charring reagent detected all of the phospholipids as brown-black zones on a white background, but, as seen in Table 1, some compounds could not be detected in one or more of the solvent systems at the concentration spotted. Lysolecithin

TABLE 2

Phospholipid R_f Values

Solvent system #		1	2	3	4
		Rf values (cm X 100)			
Phospholipid					
Phosphatidylcholine		36	49	64	5
Phosphatidic acid		35	37	78	nd
Phosphatidylethanolamine		54	60	80	46
Lysolecithin		17	nd	34	12
Sphingomyelin		25	33	58	8
Cerebrosides	1)	62	60	80	84
	2)	60	56	78	80
	3)	57	54	77	78
Sulfatides	1)	36	28	69	77
	2)	34	28	70	77
Phosphatidylserine		29	28	66	25
Phosphatidylinositol		24	20	68	24
Phosphatidylglycerol		37	32	70	nd
Sphingolipid mix	a) cerebrosides	62	56	80	81
	b) sulfatides	36	28	68	76
	c) sphingomyelin	25	29	58	9
Polar Lipid Mix	a) cholesterol	85	76	90	90
	b) phosphatidylethanolamine	55	56	80	45
	c) phosphatidylcholine	36	42	68	10
Cardiolipin		44	40	81	60
Lysophosphatidylethanolamine		nd	nd	82	nd
Monogalactosyldiglyceride		70	69	84	84
Digalactosyldiglyceride		49	42	70	60

nd = not detected

was detected at the 5 ug level as an individual standard, but could not be detected at the 250 ng level in the polar lipid standard.

Table 2 contains R_f values for the four systems that gave the best results for separation and detection of the phospholipids studied. Three of these four successful mobile phases contained different proportions of chloroform, methanol, and water, and the fourth included hexane and acetic acid in addition to these three components. The other four systems in Table 1 provided either poor resolution and/or detection on the HP silica gel plate used. Figure 1 illustrates a typical plate developed whith System 1, which

was judged to be the best for overall phospholipid analysis by silica gel HPTLC. The data in Table 1 can be used to choose among the four mobile phases in order to carry out the separation or quantification of a single compound or a mxture. Separations that cannot be achieved by one-dimensional HPTLC with any of the four systems may be possible by two-dimensional development.

Extract of DGGs from B. glabrata snails was included on each plate to test how the solvent systems would resolve the phospholipids in a biological sample. Using two-dimensional TLC, Thompson (10) showed that the major phospholipids in the DGG of B. glabrata maintained on a lettuce-Tetramin diet were phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine. Our unidimensional HPTLC results with solvent system 1 (Figure 1) confirm Thompson's findings.

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