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ANALYSIS OF LIPIDS BY ONE-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A high-efficiency silica gel, (type HLF) thin-layer chromatography plate (HETLC), linear high-performance thin-layer chromatography plate (HPTLC) and densitometry method has been devised in order to resolve the major lipid classes obtained from rat brain tissues. This methodology, which has largely overcome prior problems, enhances the opportunity for assessing the glycerophospholipid and glycolipid compositions of tissues. DEAE-Sephadex column chromatography was used to separate the crude lipids extract into neutral and acidic lipid fractions. The lipid fractions were then spotted on separate HPTLC and HETLC plates and chromatographed in one dimension using one solvent system. Quantitation was by *in situ* densitometry with the absolute quantity of the lipid classes determined from co-chromatographed standards. Sensitivity was increased by using cupric sulfate reagent, which was found to be more sensitive than the conventional cupric acetate reagent. This method is applicable to a broader separation of lipid classes and has improved sensitivity.

INTRODUCTION

An accurate method for extraction, separation, and analysis of different lipid classes is important in biological studies, especially those relating to membrane biochemistry. The difficulty of resolving these problems is evident from the numerous methods described in the literature and from the lack of suitability of any one method to resolve major classes of lipids.

The procedure of Folch *et al.*[1] is by far the most frequently quoted method for preparing lipid samples from tissues. It has not proved suitable in all circumstances, but its modification, proposed by Ways and Hanahan[2] is more broadly applicable. We sought to develop a sensitive and reliable method for the extraction of lipids by a further modification of the Folch procedure followed by thin-layer chromatography (TLC) on silica gel high-performance (HP) plates and high-efficiency (HE) uniplates, chromatographed in a one-dimensional system, visualized by a cupric sulfate reagent and quantified by densitometry.

In situ densitometry of thin-layer chromatograms was first used by Privett *et*

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al.[3]. They separated acylglycerols by TLC and quantitated them after charring with sulfuric acid. Many methods have subsequently been developed to analyze certain, but not all, of the major lipid classes. Some methods are limited to the neutral lipid classes[4–7] or the glycosphingolipid or phospholipid classes[8–15], while others include some, but not all, classes of both the neutral and phospholipids[16,17]. Complete separation of the major lipid groups usually requires two-dimensional TLC development, which suffers the disadvantage of being able to handle only one sample per plate.

A growing number of methods have been developed to attempt one-dimensional TLC for the resolution of lipids. Some of these methods use two or more development systems for TLC[16–19]. Chemical pretreatment of the TLC plate also has been used for complete separation of the major lipid classes[20,21]. Ando and co-workers[22,23] developed a HPLTC method which separates the major neutral and phospholipid classes, with the exception of PI and PS*. Also, sulfatides which were not found in the tissues they analyzed, co-migrate with PE in their systems. Macala *et al.*[16] also separated the major neutral and phospholipids on HPTLC with two solvent systems. However, using their methodology in our laboratory we were unable to resolve PG, LPC and PA standards. Although Heape *et al.*[24] reported a one-dimensional TLC technique which allows the resolution of the major polar lipids, when this technique was applied in our laboratory PG co-migrated with PE in a test HPTLC plate and PG co-migrated with SULF in a test HETLC plate.

We developed a lipid analysis system which permits separation of the major classes and sensitive quantification. We modified the lipid extraction procedure of Folch *et al.*[1], and Ways and Hanahan[2] by the addition of several steps. We modified the chromatographic procedure of Heape *et al.*[24] by using DEAE-Sephadex column chromatography to first separate the total lipids extract into neutral and acidic lipid fractions which permitted the further separation of the major lipid classes (LPC, SPh, PC, PI, PS, PE, PA, PG, SULF, and CB) on HPTLC and HETLC for quantification by densitometry. hR_F values were calculated for both HPTLC and HETLC plates using three different solvent systems. This procedure affords a sensitive and accurate means of analyzing the lipid compositions of brain tissues.

EXPERIMENTAL

Materials

All phospholipid, and glycolipid standards were obtained from Avanti Polar Lipid (Birmingham, AL, U.S.A.), with the exception of oleyl alcohol (as internal standard) which was from American Scientific Products (Grand Prairie, TX, U.S.A.). DEAE-Sephadex (A-25) was from Pharmacia Fine Chemicals (Milwaukee, WI, U.S.A.). HPTLC plates (silica gel 60, 0.20-mm layer thickness, 10 × 10 cm) were from Curtin Matheson Scientific (Dallas, TX, U.S.A.), and HETLC plates (preadsorbent high-efficiency silica gel, type HLF, 10 × 10 cm plates) were received from

* Abbreviations: LPC = lysophosphatidylcholine; SPh = sphingomyelin; PC = choline phospholipids; PE = ethanolamine phospholipids; PS = serine phospholipids; CB = cerebrosides; SULF = sulfatides; PG = phosphatidylglycerol; PA = phosphatidic acid; IS = internal standard; PI = inositol phospholipids. Acidic lipids refer to PI, PS, SULF, PG, and PA; all other lipids are collectively referred to as non-acidic lipids.

Analtech (Newark, DE, U.S.A.). All reagents and solvents were analytical reagent grade.

Standard lipid mixtures

Each standard lipid was dissolved in a specific volume of chloroform–methanol (2:1, v/v). Seven different concentrations of standard lipid mixtures were then prepared (200, 600, 1000, 2000, 3000, 5000, 10 000 ppm) for each of the following lipids: LPC, PC, SPh, PE, CB, PI, PS, SULF, PA, and PG. Similar standard lipid mixtures were prepared for the non-acidic and acidic lipids. To all standard lipid mixtures, oleyl alcohol was added to a final concentration of 1 μg/ul as an internal standard.

Biological samples

The biological samples analyzed consisted of lipid extracts from rat brain. Approximately 0.25–0.5 g of whole rat brain tissue was homogenized for 1 min with 0.5 ml of water and the process continued for a further 2 min with 5 ml of methanol and 1 min with 10 ml of chloroform. The mixtures were filtered and the solid residue resuspended in 15 ml chloroform–methanol (2:1, v/v) and homogenized for 3 min. After filtering, the solid was washed once more with chloroform (10 ml) and once with methanol (5 ml). The combined filtrates were transferred to a measuring cylinder and one-quarter of the total volume of 0.88% potassium chloride in water was added; the mixture was shaken thoroughly in a glass separatory funnel and allowed to settle overnight. The lower layer was separated, one-quarter of the volume of the lower layer of water–methanol (1:1, v/v) was added and the washing procedure repeated. The bottom layer containing the purified lipids was withdrawn and concentrated under nitrogen gas, and the residue was dissolved to a suitable volume (*e.g.*, 10 ml) in chloroform–methanol (2:1, v/v) and then frozen[1]. Aliquots of the lipids were separated into neutral and acidic fractions by ion-exchange chromatography on a DEAE-Sephadex column[15]. The lipids and standards were dissolved in chloroform–methanol–water (30:60:8, v/v/v) when applied to the Sephadex column. The neutral fraction was eluted with the same solvent and the acidic fraction was eluted with chloroform–methanol–0.8 *M* aqueous sodium acetate (30:60:8, v/v/v). The acidic lipid fractions were further treated, to remove the sodium acetate, prior to chromatogram plates[15]. To all above organic compounds 0.05% (w/v) antioxidant (2,6-di-*tert.*-butyl-*p*-cresol) was added.

Sample application and chromatographic separation

HPTLC and HETLC plates were prewashed once in chloroform–methanol (1:1, v/v) and then dried and washed again in each test solvent system. Plates were dried after the second wash and activated at 100°C for 15 min and cooled in a vacuum desiccator. The standard lipids and the brain lipid extracts were spotted onto the HETLC and HPTLC plates. The spotting volume was 1 μl for all standard lipids and the brain lipid extracts. test plates were chromatographed in TLC tanks lined with filter paper. Solvent systems were equilibrated several hours before use. The lipids were chromatographed with one of the following solvent systems: A1, denaturated ethanol–chloroform–ammonium hydroxide (50:6:6, v/v/v); A2, methyl acetate–*n*-propanol–chloroform–methanol–0.25% aqueous potassium chloride (25:25:28:10:7, v/v/v/v/v); B1, chloroform–methanol–acetic acid–formic acid–water (35:15:6:2:1,

v/v/v/vv/v); B2, hexane–diisopropyl ether–acetic acid (65:35:2, v/v/v); C, methyl acetate–*n*-propanol–chloroform–methanol–0.25% aqueous potassium chloride (25:25:28:10:7, v/v/v/v/v). The chromatograph was stopped when the solvent front ascended to about 3 cm above the bottom edge of the plate in solvent A1, 5 cm in solvent B1, and to the top of plate in solvent C. Following development, excess solvent was evaporated in a fume hood for 15 min. Lipids (acidic, non-acidic, and mixtures) were then chromatographed in solvent A2 and B2 until the solvent front ascended to the top of the plate. After development, the chromatograms were again dried in a fume hood to remove the solvent. Residual solvent was removed by heating at 170°C for 3 min immediately prior to charring. Plates were either sprayed with 10% (w/v) cupric sulfate or 3% (w/v) cupric acetate in 8% (v/v) phosphoric acid solution and heated at 180°C for 15–20 min[2]. Plates were generally scanned immediately after heating, or stored at −20°C and scanned within 24 h.

Densitometry and quantitation

The developed plates were scanned from the origin to the solvent front at 525 nm in the absorbance mode using an auto scanner Flur–VIS densitometer (Helena Labs, Beaumont, TX, U.S.A.). The integrated area was computed by a Helena Quick Quant II (Helena Labs.). Each lane was scanned three times and the average values for the three scans were determined. For the quantitative analyses, we used a technique similar to that described by Macala *et al.*[16].

RESULTS

HETLC and HPTLC plates, with their corresponding densitometric chromatograms are shown for the mixture (Fig. 1), the non-acidic lipid fraction (Fig. 2)

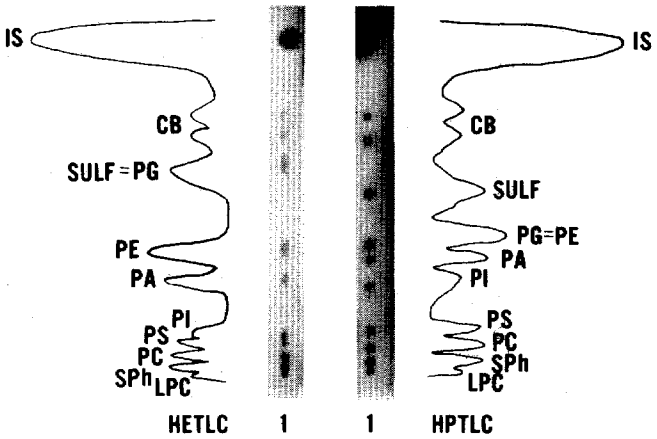

Fig. 1. HETLC and HPTLC chromatograms of mixture standard lipids, with densitometric chromatograms. The lipids were applied in 1 μl of chloroform–methanol (2:1, v/v) contained 0.6 μg each of lysophosphatidylcholine (LPC), sphingomyelin (SPh), choline phospholipids (PC), serine phospholipids (PS), inositol phospholipids (PI), phosphadatidic acid (PA), phosphatidylglycerol (PG), ethanolamine phospholipids (PE), sulfatides (SULF), cerebrosides (CB), and 1 μg of internal standards (IS). The lipids were applied in 1 μl of chloroform–methanol (2:1, v/v). Solvent system: methyl acetate–propanol–chloroform–methanol–0.25% aqueous potassium chloride (25:25:28:10:7, v/v/v/v/v). The charring reagent was 8% cupric sulfate in 8% phosphoric acid.

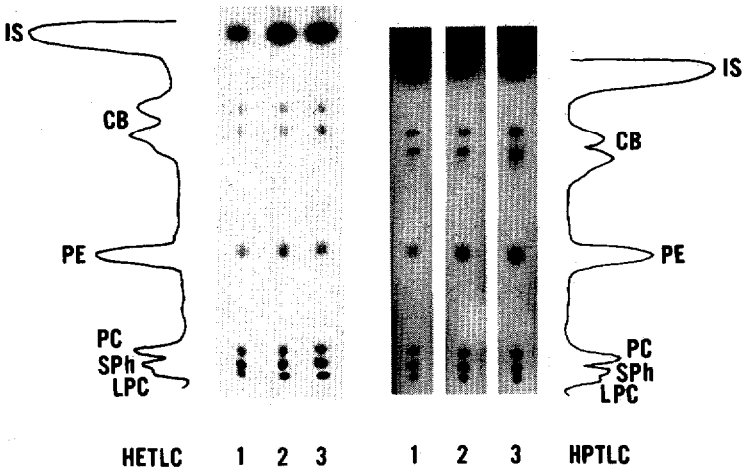

Fig. 2. HETLC and HPTLC chromatograms of non-acidic standard lipids, with densitometric chromatograms of lanes 1. Lanes 1, 2, and 3 respectively, contained 0.6, 1 and 2 μg each of lysophosphatidylcholine (LPC), sphingomyelin (SPh), cholinephospholipids (PC), ethanolamine phospholipids (PE), cerebrosides (CB), and 1 μg of internal standard (IS). The lipids were applied in 1 μl of chloroform–methanol (2:1, v/v). Solvent system: methyl acetate–*n*-propanol–chloroform–methanol–0.25% aqueous potassium chloride (25:25:28:10:7, v/v/v/v/v). The charring reagent was 8% cupric sulfate in 8% phosphoric acid.

and the acidic lipid fraction (Fig. 3) of the standard lipids. Standard curves for each non-acidic and each lipid class indicate that each lipid class had a different densitometric response, which was non-linear below the range of 600 ppm (0.6 μg). Also, concentration of standard lipids above the range of 3000 ppm resulted in overlapping of LPC and SPh in non-acidic lipids and PS and PI in acidic lipids.

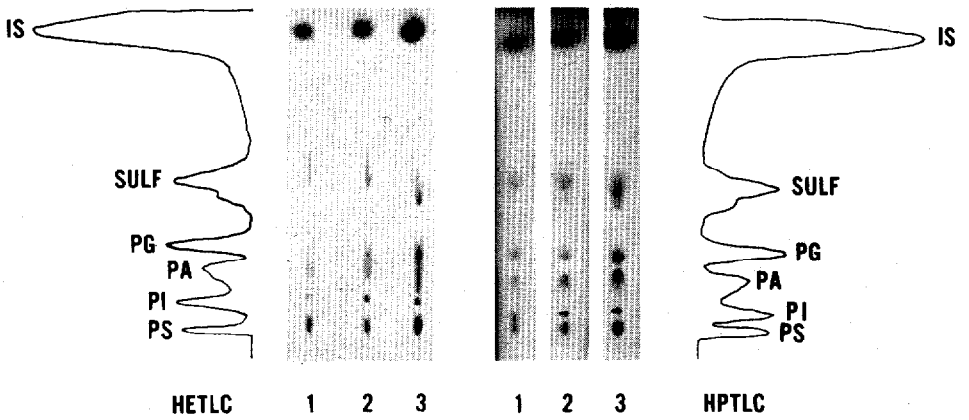

Fig. 3. HETLC and HPTLC chromatograms of acidic standard lipids, with densitometric chromatograms of lanes 1. Lanes 1, 2, and 3 respectively, contained 0.6, 1 and 2 μg each of serine phospholipids (PS), inositol phospholipids (PI), phosphatidic acid (PA), phosphatidylglycerol (PG), sulfatides (SULF), and 1 μg of internal standards (IS). The lipids were applied in 1 μl of chloroform–methanol (2:1, v/v). Solvent system: methyl acetate–*n*-propanol–chloroform–methanol–0.25% aqueous potassium chloride (25:25:28:10:7, v/v/v/v/v). The charring reagent was 10% cupric sulfate in 8% phosphoric acid.

The hR_F values of each of the individual lipid classes before the samples separated into the acidic and non-acidic fractions are given in Table I. Each concentration of standard lipid mixtures was spotted in duplicate on three different HPTLC and HETLC plates when chromatographed for each solvent system.

TABLE I

hR_F VALUES OF RAT BRAIN LIPIDS AND STANDARD LIPIDS FROM HPTLC AND HETLC BY THE DIFFERENT SOLVENT SYSTEMS

HETLC and HPTLC using the solvent systems A, B, and C as described in the Experimental section. Number of different measurements, 108; number of either HPTLC or HETLC Plates, 3. S.D. = Standard deviation.

Lipid	*HETLC hR_F (mean ± S.D.)*			*HPTLC hR_F (mean ± S.D.)*		
	A	*B*	*C*	*A*	*B*	*C*
LPC	8 ± 0.1	*	6 ± 0.83	9 ± 1.0	*	6 ± 0.71
SPh	9 ± 0.3	2 ± 0.41	11 ± 0.71	4 ± 0.01	2 ± 0.40	12 ± 0.82
PC	13 ± 0.71	4 ± 0.75	14 ± 0.71	6 ± 0.2	4 ± 0.8	11 ± 1.0
PI	19 ± 0.71	2 ± 0.5	19 ± 0.7	25 ± 0.6	2 ± 0.5	19 ± 0.7
PS	15 ± 0.71	3 ± 0.71	15 ± 0.65	15 ± 1.1	3 ± 0.6	15 ± 0.6
PE	29 ± 1.1	9.3 ± 1.0	31 ± 1.0	17 ± 1.0	9 ± 1.25	31 ± 2.1
PA	10 ± 0.82	*	22 ± 1.2	8 ± 0.71	*	26 ± 1.0
PG	40 ± 3.0	*	38 ± 2.0	40 ± 3.1	*	31 ± 3.1
SULF	50 ± 1.9	3 ± 0.71	52 ± 1.0	18 ± 0.1	30 ± 0.6	41 ± 1.1
CB	50 ± 1.9	14 ± 2.4	52 ± 2.0	18 ± 0.1	13 ± 2.0	51 ± 1.0
	56 ± 1.0	20 ± 1.6	59 ± 1.0		17 ± 0.94	57 ± 0.85
IS	90 ± 0.2	89 ± 0.1	91 ± 0.1	90 ± 0.1	89 ± 0.1	91 ± 0.1

* Lipid was not resolved at this solvent system.

Data for each lipid class, expressed as a percent of total lipid, and total lipid, expressed on an absolute basis, are shown in comparison to data reported by other investigator (Table II). Total lipids were extracted from the whole brain of a single rat. The extract was adjusted to a final concentration of chloroform–methanol–water (30:60:8, v/v/v) when applied to the Sephadex column. Aliquots corresponding to 0.2–0.5 g of tissues wet weight were taken, in triplicate. After isolating the non-acidic and acidic lipids for 18 samples, each sample was spotted in duplicate on three different HPTLC plates and the individual lipid classes were quantitated (18 determinations for each tissue wet weight). The results for all determinations (N = 324) are shown in Table II.

DISCUSSION

We examined the original solvent systems B and C proposed by Macala *et al.*[16] and Heape *et al.*[24], respectively, as possible mobile phases for separation of rat brain crude lipids extracted by a modification of the Folch procedure. The protocol was validated by analysis of various standard lipids (LPC, PC, PE, Sph, CB, PG, PA, PI, PS, and SULF). As expected, in solvent system B, PG, LPC, and PA were

TABLE II

ANALYSIS OF RAT WHOLE BRAIN LIPIDS ON HETLC AND HPTLC USING SOLVENT SYSTEM C

Individual lipid classes are expressed as a mean of percent of total lipids ± standard deviation (S.D.). ww = Wet weight.

Lipid	*Method*		*Literature*	
	HETLC	*HPTLC*	*Ref. 16*	*Ref. 26*
C*	No***	No***	18.6 ± 0.2	22.2 ± 0.3
LPC**	1.98 ± 0.4	2.33 ± 0.3	§	§
SPh	3.80 ± 0.2	4.00 ± 0.4	2.8 ± 0.0	4.3 ± 0.4
PC	22.50 ± 0.4	20.42 ± 0.4	22.6 ± 0.3	24.1 ± 0.2
PI	3.85 ± 0.3	4.61 ± 1.0	2.4 ± 0.0	2.7 ± 0.4
PS	12.60 ± 0.5	9.65 ± 0.8	14.6 ± 0.4	7.0 ± 0.2
PE	24.50 ± 0.5	22.04 ± 0.8	24.1 ± 0.2	19.8 ± 0.5
PA**	4.82 ± 1.0	6.83 ± 1.0	§	§
PG**	§§	§§	§	§
Sulf	6.25 ± 0.3	8.23 ± 0.5	3.6 ± 0.0	3.6 ± 0.1
CB	18.50 ± 0.5	20.00 ± 0.5	11.3 ± 0.1	11.9 ± 0.2
Total lipids	80.00 ± 0.8 (mg/g ww)	82.2 ± 1.0 (mg/g ww)	74.3 ± 0.7 (mg/g ww)	84.5 (mg/g ww)

* Cholesterol.
** Not routinely detected in normal brain tissue.
*** At this time lipid was not analyzed.
§ Data was not reported.
§§ Lipid was not detected.

not resolved in standard lipids. Also our results showed SULF, whether originating in the crude lipid extract, the acidic lipid fraction, or the standard lipid mixture, always co-migrated with PS (hR_F = 3.0 ± 0.71) (mean ± standard deviation), and remained near the origin, leading to overlap of the corresponding peaks obtained by densitometric scanning. This result was similar for both the HPTLC and HETLC plates (Table I). We used solvent system C to resolve these problems. PS and SULF, which co-migrated with the previous solvent system, were resolved by solvent system C. However, solvent system C did not adequately separate PG from PE in HPTLC and CB from SULF in HETLC plates.

This observation led us to carry out a systematic study of the effects of other solvent systems on the migration of the various lipids in order to try to resolve the co-migrating PG from PE in HPTLC, and PG from SULF in HETLC. We used the solvent system A which resulted from two mobile phases: (1) proposed by Dugan[25] and (2) by Heape *et al.*[24]. Under these conditions, the system moved the PG with good resolution, well ahead of PE. Also, all of the lipids obtained substantially good hR_F values, while the rate of migration of PA was retarded. However, as expected, an ethanol, ammonium hydroxide, and chloroform mixture helped to provide a good separation of PG from PE and SULF, with PC and SPh. Furthermore, PS and PI were pulled from the origin with good separation. CB and SULF which were resolved

with the previous solvent system, co-migrated with solvent system A. However, all of the above problems were solved by an initial separation of the lipids into non-acidic and acid lipid fractions by DEAE-Sephadex column chromatography[26], separating PG, and SULF (acidic) from PE and CB (non-acidic).

The well defined peaks obtined by densitometric scanning of either HPTLC or HETLC plates allow us to use solvent system A or C for the accurate quantitative analysis of rat brain lipids by densitometry, *in situ,* on the chromatographic plates. Moreover, the contamination risk is very much reduced compared with existing one-dimensional techniques, since we separated the total lipids into acidic and non-acidic lipids. If an analysis of the neutral lipids which migrate to the solvent front is desired, this can be accomplished as follows. The first elution, using the solvent system C, is allowed to continue for a distance of 6.0 cm (origin to solvent front). A second elution, using the solvent system hexane diisopropyl ether–acetic acid (75:23:2, v/v/v), in the same direction as the first elution, is then carried out to the top of the chromatoplate. This second elution, allows the resolution of cholesterol, free fatty acid, diacylglycerols, triacylglycerols and cholesterol esters[24].

Sensitivity was also improved by using the cupric sulfate reagent[27], in conjunction with HPTLC and HETLC. In our study, we found that the percent values of the PI, PC and PE, when charred with cupric sulfate reagent, were generally two times more sensitive than cupric acetate, which may be the result of the reaction of the cupric sulfate reagent with both the saturated and the unsaturated fatty acid moieties of lipids as reported by Touchstone *et al.*[27]. For the lipids examined other than PI, PC and PE, sensitivity was approximately the same for both reagents. With the present method, approximately 0.2 μg of each lipid class was easily quantitated.

In addition to improved sensitivity we found some differences between the percent distribution of the individual lipid classes when compared to the literature values (Table II). The percent values for PI, SULF, and CB were about two times higher than literature values, while PS, PE, PC, SPh wer in close agreement with literature values. Also, the data we obtained by densitometry for the individual lipid classes in HPTLC and HETLC were similar, which suggests to us either HETLC or HPTLC can be used to resolve LPC, SPh, PC, PE, PI, PS, CB, PG, PA, and SULF. Also, it should be noted that in several cases we found LPC and PA, which are not found in normal brain tissue, were detected (Table II).

In conclusion, we have developed a method to analyze the major lipid classes in rat brain. This method adequately separates the major lipids (LPC, SPh, PC, PE, CB, PG, PA, PI, PS, and SULF) groups which are readily identified by their hR_F values, and permits identification and quantification of these groups either on preadsorbent silica gel HETLC or HPTLC. This method is sensitive and rapid. It requires only a single column chromatography step to separate the total lipids into non-acidic and acidic fractions, followed by one-dimensional HPTLC or HETLC using only one solvent system. This is followed by *in situ* densitometry, to quantitate each lipid class, using external standards and an internal standard. The procedure should be useful in determining the lipid compositions of small amounts of tissues or in samples where the total lipid content is low.

We have initiated this investigation into the analysis of lipid classes in rat brain in order to provide the techniques for a study of lipids in the brain stems of sudden infant death syndrome (SIDS) victims. The SIDS study is currently underway. Four-

ier transfrom infrared, gas chromatography, and high-performance liquid chromatography techniques are also being developed for lipid structural identification.

REFERENCES

1 J. Folch, M. Lees and G.A. Stanley, *J. Biol. Chem.*, 226 (1975) 497–509.
2 P. Ways and D. J. Hanahan, *J. Lipid Res.*, 5 (1964) 318–322.
3 O. S. Privett, M. L. Blank, D. W. Codding and E. C. Nickell, *J. Am. Oil Chem. Soc.*, 42 (1965) 381–393.
4 V. P. Skipski, A. F. Smolowe, R. C. Sullivan and M. Barclay, *Biochem. Biophys. Acta*, 106 (1965) 386–396.
5 J. L. Hojnacki, R. J. Nicolosi and K. C. Hayes, *J. Chromatogr.*, 128 (1976) 133–139.
6 J. J. Kabara and J. S. Chen, *Anal. Chem.*, 48 (1976) 814–817.
7 J. Bitman, O. L. Wood and J. M. Ruth, *J. Liq. Chromatogr.*, 4 (1981) 1007–1021.
8 V. P. Skipski, R. F. Peterson and M. Barclay, *Biochem. J.*, 90 (1964) 374–378.
9 A. A. Pappas, R. E. Mullins and R. H. Gadsden, *J. Clin. Chem.*, 28 (1982) 209–211.
10 O. S. Privett, K. A. Dougherty and J. D. Castell, *Am. J. Clin. Nutr.*, 24 (1971) 1265–1273.
11 W. W. Young, Jr. and C. A. Borgman, *J. Lipid Res.*, 27 (1986) 120–124.
12 J. F. Pernes, Y. Nurit and M. De Heauline, *J. Chromatogr.*, 181 (1980) 254–258.
13 L. Gustavsson, *J. Chromatogr.*, 375 (1986) 255–266.
14 J. K. G. Kramer, R. C. Fouchard and E. R. Farnworth, *J. Chromatogr.*, 351 (1986) 571–573.
15 J. C. Touchstone, J. C. Chen and K. M. Beaver, *J. Lipids*, 15 (1980) 61–62.
16 L. J. Macala, R. K. Yu and S. Ando, *J. Lipid Res.*, 24 (1983) 1243–1250.
17 F. M. Helmy and M. H. Hack, *J. Chromatogr.*, 374 (1986) 61–72.
18 A. R. N. Eberendu, K. E. Daugherty and B. J. Venables, *LC, Liq. Chromatogr. HPLC Mag.*, 13 (1985) 424–428.
19 P. R. Gentner, M. Bauer and I. Dieterich, *J. Chromatogr.*, 206 (1981) 200–204.
20 P. C. Painter, *Clin. Chem.*, 26 (1980) 1147–1151.
21 J. B. Fine and H. Sprecher, *J. Lipid. Res.*, 23 (1982) 660–663.
22 S. Ando, K. Kon, Y. Tanaka, S. Nagase and Y. Nagai, *J. Biochem.*, 87 (1980) 1859–1862.
23 S. Ando, K. Kon and Y. Tanaka, in M. Kates and A. Kuksis (Editors), *Membrane Fludity Biophysical Techniques and Cellular Regulation*, Human Press, Clifton NJ, 1980, pp. 43–55.
24 A. M. Heape, H. Juguelin, F. Boiron and C. Cassagne, *J. Chromatogr.*, 322 (1985) 391–394.
25 E. A. Dugan, *Analysis of Phospholipid by ONe-Dimensional Thin-Layer Chromatography*, Analtech Inc., Newark, DE, 1985.
26 W. T. Norton and S. E. Poduslo, *J. Neurochem.*, 21 (1973) 759–773.
27 J. C. Touchstone, S. S. Levin, M. F. Dobbins and P. C. Beers, *J. Liq. Chromatogr.*, 6 (1983) 179–192.