Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 813 (2004) 159–166
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Variable recoveries of fatty acids following the separation of lipids on commercial silica gel TLC plates
Selective loss of unsaturated fatty acids on certain brands of plates

Jennifer M. Sowa, Papasani V. Subbaiah*

Department of Medicine and Department of Biochemistry & Molecular Genetics, University of Illinois at Chicago, 1819 South Wolcott, M/C 797, Chicago, IL 60612, USA

Received 5 July 2004; accepted 16 September 2004
Available online 22 October 2004

Abstract

Since we recently noticed poor recoveries of unsaturated fatty acids (UFA) when the parent lipids were first separated on TLC plates, we investigated the source of this error by examining several variables, including the brand of TLC plate, nature of the lipid, and conditions of methylation. Of the five commercial brands of plates used, two (Baker and Whatman) showed loss of UFA, and three (Alltech Hardlayer, Alltech Softlayer, and Merck) did not. This loss occurred in both neutral and phospholipids, did not affect saturated acids, and was independent of the methylation reagent used. No loss occurred, however, if the lipids were eluted from the silica gel before methylation, indicating that the loss is due to oxidation of UFA in presence of certain brands of silica gel. These results show that some brands of TLC plates may be unsuitable for lipid analysis, if the aim is to determine the fatty acid composition by GC using direct methylation.

Keywords: Thin-layer chromatography; Commercial silica gel plates; Unsaturated fatty acids; Phospholipids; Cholesteryl esters; Free fatty acids; Methylation; Fatty acid methyl esters; Gas liquid chromatography; Oxidative degradation

1. Introduction

Thin layer chromatography (TLC) on silica gel plates is probably the most commonly used chromatographic procedure for the separation of lipid classes. Although HPLC provides a more efficient separation of lipid classes, the TLC method enjoys several advantages including the lower cost, less rigorous sample preparation, the ability to analyze multiple samples simultaneously, and the ease of visualization. Most laboratories now use commercially prepared plates rather than prepare them in house, because the commercial plates have the uniformity in thickness, stability of the silica gel layer, and greater reproducibility of separations. We have been using commercial silica gel TLC plates for several years for the separation and quantitation of phospholipids [1–3], as well as for the subsequent analysis of molecular species of phospholipids by HPLC [4–6], or analysis of fatty acid composition by GC [7]. For the analysis of their fatty acid composition, the various lipids separated by TLC are commonly methylated directly in the presence of the silica gel [8–10]. Although we have successfully used these methods for several years, we noticed in recent months that the recovery of unsaturated fatty acids was dramatically reduced following the separation of lipid classes by TLC, direct transmethylation of fatty acids in presence of the silica gel, and subsequent GC analyses. A systematic search for the source of this loss was therefore undertaken by examining each step of the analysis, and by using different brands of commercially available silica gel TLC plates. The objective of this study was to determine whether various commercial TLC plates differ in their effect on the recovery of

* Corresponding author. Tel.: +1 312 996 8212; fax: +1 312 413 0437.
E-mail address: psubbaia@uic.edu (P.V. Subbaiah).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.09.026

unsaturated fatty acids, and to identify the possible cause of this loss.

2. Experimental

2.1. TLC plates

Five commercial brands of silica gel TLC plates were compared in this study: Alltech soft layer (Adsorbosil Plus 1, Lot Nos. 22403 and 33803, 20 cm × 20 cm), Alltech hard layer (Adsorbosil Plus 1, Lot Nos. 14004 and 02704, 20 cm × 20 cm), EM Science (Lot Nos. OB460569 and OB330789, 10 cm × 20 cm), J.T. Baker (Lot No. 38439, 10 cm × 20 cm), and Whatman (Partisil K6, Lot Nos. 31043845 and 31048265, 10 cm × 20 cm). All plates were 250 μm in thickness, were purchased from VWR Scientific (Chicago, IL), and were used within one month of purchase. There was no difference in the results obtained from the two different lots of plates from the same company.

2.2. Lipid standards

Synthetic phosphatidylcholines (PC) (17:0–17:0 PC, 16:0–18:1 PC, 16:0–18:2 PC, and 16:0–20:4 PC) were obtained from Avanti Polar Lipids (Alabaster, AL). The fatty acid purity of the PCs was analyzed by GC, and all PCs were found to be >98% pure. Cholesteryl esters (CE) (17:0, 18:1, 18:2, and 20:4) were purchased from Sigma–Aldrich (St. Louis, MO) and free fatty acids (17:0, 18:1, 18:2, 20:4) were purchased from NuChek (Elysian, MN). All lipid samples were used without further purification. All the solvents for extraction and TLC were purchased from Fisher Scientific (Chicago, IL).

2.3. TLC separation of lipids

All plates were scored with 2 cm lanes, and heated at 110 °C for 30 min before use. The plates were allowed to cool to room temperature before spotting the samples. The PC samples (0.1 μmol + 0.1 μmol of 17:0–17:0 PC as internal standard) were spotted in 100 μl of chloroform. Egg PC (100 μg) was used as a visualization standard on a separate lane for identification of PC bands. The samples were run with the solvent system of chloroform:methanol:water (65:25:4, v/v/v) until the solvent front was 2 cm from the top of the plate. The plate was air dried for 5 min in a fume hood, and the lane containing the standard PC was exposed to iodine vapors after covering the lanes of experimental samples with a clean glass plate. The area corresponding to PC standard was scraped and immediately transmethylated as described below.

Free fatty acids (FFA) and CE were chromatographed separately on the plates using the solvent system of hexane:diethyl ether:acetic acid (70:30:1, v/v/v), using 17:0 FFA and cholesteryl-17:0 as internal standards, respectively. Free oleic acid or cholesteryl oleate (100 μg each) was used as visualization standard on a separate lane. After air-drying the plate for 5 min in a fume hood, the lane containing the standards was exposed to iodine vapors, and the band in the sample lane corresponding to free fatty acid or cholesteryl ester was scraped and methylated directly.

2.4. Methylation and GC analysis

All lipid samples were transmethylated routinely by using BF_3 in methanol (Alltech). To the scrapings from the TLC plate or the dried lipid samples, 1 ml of BF_3–methanol was added, and the samples were heated at 90 °C for 1 h after flushing the tube with N_2 and capping with a Teflon-lined cap.. The samples were cooled to room temperature, 1 ml water was added, and the methyl esters were extracted twice with 2 ml hexane.

The pooled hexane extracts were evaporated under N_2 and analyzed on a Shimadzu GC 17A chromatographic system equipped with a flame ionization detector, with the following conditions. An Omegawax (30 m × 0.25 mm, Supelco) column was used with hydrogen (1.2 ml/min) as carrier gas, and a split ratio of 50:1. N_2 was used as the makeup gas. The temperature programming was as follows. Initial temperature was 175 °C (8 min), raised to 200 °C at the rate of 4.5 °C/min and kept at 200 °C for 8 min, raised to 225 °C at the rate of 6 °C/min, and maintained at this temperature for 15 min. In the case of CE samples, an additional step was included at the end, where the temperature was raised to 270 °C (20 °C/min) and maintained for 15 min, to elute free sterols or other contaminants that would otherwise interfere in the subsequent runs. The area percentages of the fatty acids were calculated online with the EZChrom software (Shimadzu). The percent recovery of each fatty acid was calculated relative to that of 17:0 internal standard.

3. Results and discussion

3.1. Recovery of PC fatty acids after TLC separation on various commercial plates

Samples of three different PCs containing an unsaturated fatty acid at *sn*-2 (16:0–18:2, 16:0–18:1, and 16:0–20:4) were chromatographed on five different commercial silica gel TLC plates in presence of equimolar amounts of 17:0–17:0 PC as the internal standard, and analyzed for fatty acid composition as described in Section 2. Fig. 1 shows the gas chromatograms obtained for methyl esters of 16:0–18:2 PC (and the internal standard, 17:0–17:0 PC) after chromatography on each plate. The control sample shown was directly methylated, without running on the plate. The 18:2 peak completely disappeared from J.T. Baker and Whatman plates, whereas the peak appeared normal with the other three commercial plates. Small peaks of apparent degradation products of 18:2 were seen after separation on Baker and

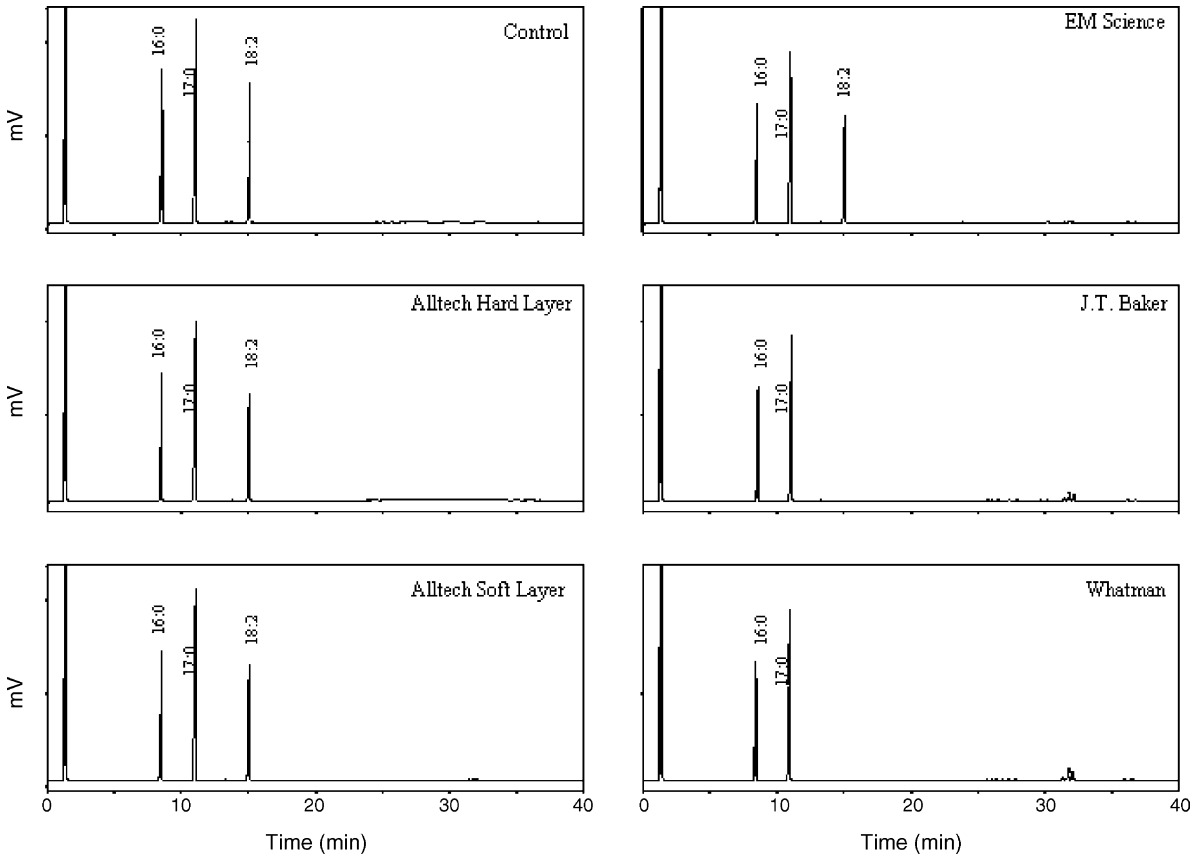

Fig. 1. Gas chromatograms of methyl esters of 16:0–18:2 PC and 17:0–17:0 PC after initial chromatography of the PC on various silica gel TLC plates. A mixture of equimolar amounts of 16:0–18:2 PC and 17:0–17:0 PC was first run on the indicated TLC plate, the methyl esters prepared from the PC spot, and analyzed on capillary GC as described in the text. The quantitative recoveries of 16:0 and 18:2 were calculated relative to those in control (unchromatographed) sample, and are shown Table 1.

Whatman plates. There was no loss of 16:0 peak on any of the plates.

Fig. 2 shows the gas chromatograms for 16:0–18:1 PC following its chromatography on various TLC plates and methylation of fatty acids. Here also there was a complete loss of 18:1 when J.T. Baker and Whatman plates were used, but no loss of 16:0 occurred. However, a new peak appeared in the samples run on Baker and Whatman plates at 31.5 min. This is apparently a degradation product of 18:1, although it needs to be confirmed by MS analysis. In the case of 16:0–20:4 PC, the 20:4 peak disappeared completely with the J.T. Baker and Whatman plates, and small peaks of degradation products were noted (Fig. 3). Since the recovery of 16:0 was close to 100% in all cases, the degradation products are from the unsaturated fatty acids. Although the degradation product was prominent in the case of 18:1, the products of degradation of 18:2 and 20:4 were not detected on GC in significant amounts. This is presumably because these fatty acids are degraded more extensively than 18:1, forming short chain fragments that would not be detected under the conditions used.

The quantitative recovery of each fatty acid from various PCs is shown in Table 1, relative to the recovery in control sample (not run on TLC). The amount of degradation product recovered is also shown to indicate the total recovery. The recovery of unsaturated fatty acid was satisfactory only for Alltech Hard, Alltech Soft and EM Science plates. There was some loss of 20:4 even with these plates when compared to the unchromatographed control, but there was almost a total loss of all unsaturated fatty acid with both J.T. Baker and Whatman plates. The recovery of the saturated fatty acid (16:0), however, was normal in all the plates. Since both the fatty acids of PC should be recovered in the same spot, this shows that the mobility of PC was not affected, and that the sample was not degraded during the TLC run. If the degradation had occurred on the plate during the TLC run, 16:0 would not be recovered in the area expected for PC.

3.2. Recovery of fatty acids from other lipids

In order to determine whether the loss of fatty acid was dependent upon the nature of the parent lipid, the recovery of fatty acid was determined for CE and for FFA. The solvent system used for running these samples was hexane:diethyl ether:acetic acid (70:30:1, v/v/v). Cholesteryl-17:0 or 17:0 FFA was used as the internal standard. As shown in Table 2, a significant loss of 18:1, 18:2, and 20:4 from CE occurred with J.T. Baker and Whatman plates, but not with the other three plates. Interestingly, the loss of 18:2 and 20:4 from CE was

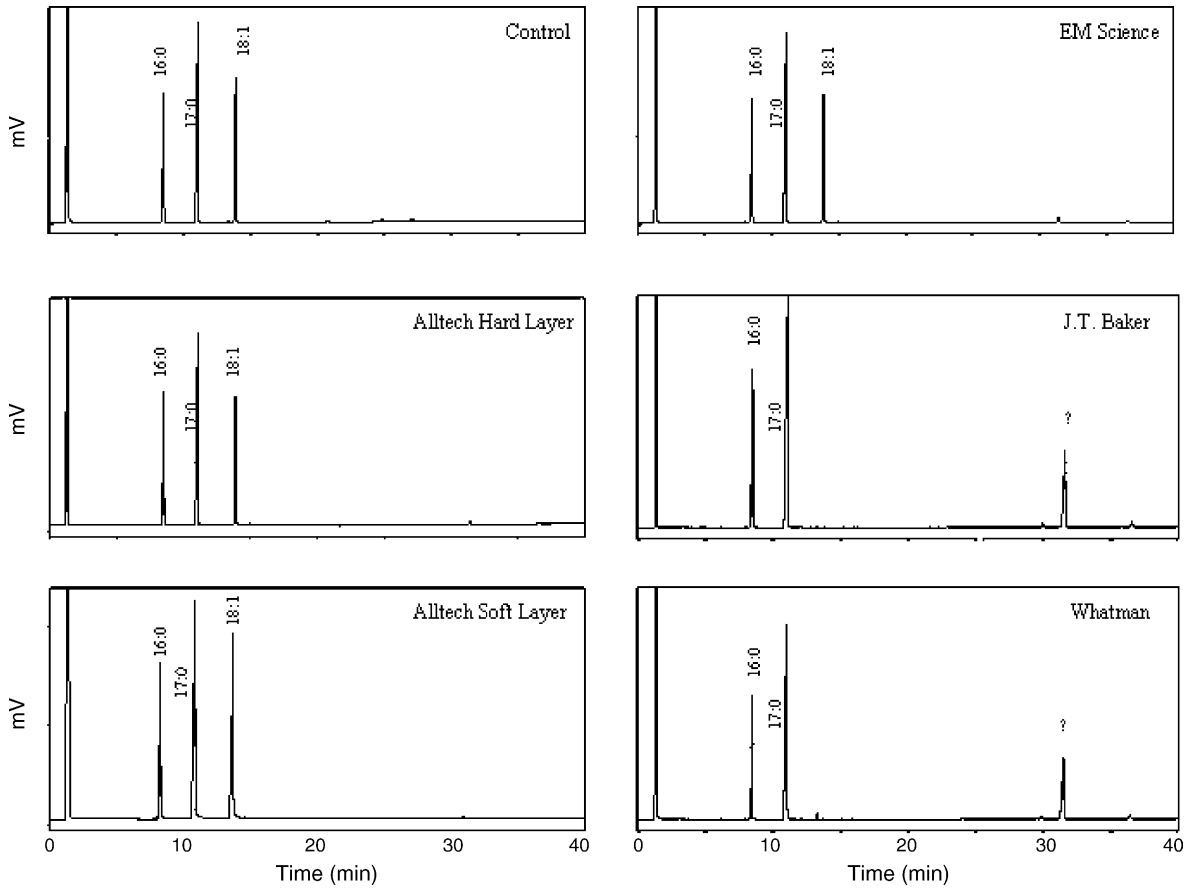

Fig. 2. Gas chromatograms of methyl esters of 16:0–18:1 PC and 17:0–17:0 PC after initial chromatography of the PC on various TLC plates. The percent recoveries of 16:0 and 18:1 were calculated relative to those in control sample, and are shown in Table 1.

less than that observed with the PC samples. The recovery of various unsaturated FFA is also shown in Table 2. Here also the loss of the unsaturated fatty acid occurred with the same brand plates as seen above for PC and CE (J.T. Baker and Whatman).

3.3. Effect of loose silica gel

To determine whether the loss of unsaturated fatty acid is due to the silica gel of the plate or due to the chromatography in the TLC solvent, we methylated two unchromatographed

Table 1
Percent recovery of fatty acids from synthetic PC species after separation on different brands of TLC plates

	Control (no TLC)	Alltech Hard	Alltech Soft	EM Science	J.T. Baker	Whatman
16:0–18:2 PC						
16:0	100.0	90.5	97.8	93.6	97.9	97.0
18:2	100.0	82.1	86.7	86.2	0.9	0.0
Degradation products	0.0	2.9	2.4	4.7	30.0	31.4
16:0–18:1 PC						
16:0	100.0	105.0	101.5	106.1	108.1	112.8
18:1	100.0	89.1	100.7	90.8	2.4	1.6
Degradation products	0.0	5.5	1.4	6.1	80.8	83.4
16:0–20:4 PC						
16:0	100.0	99.5	101.2	105.4	106.8	110.5
20:4	100.0	86.1	83.9	87.7	0.0	1.3
Degradation products	1.2	1.7	0.0	2.6	22.8	24.7

The PC samples were first run on the indicated TLC plate in presence of an internal standard (17:0–17:0 PC). The PC spot from the plate was directly methylated with BF_3–methanol, and analyzed on capillary GC, as described in the text. The recovery of individual fatty acids was calculated relative to that of 17:0 internal standard, and the recovery in the control sample (no TLC) was taken as 100%. All values shown are average of two different estimations, which differed by <10% from each other.

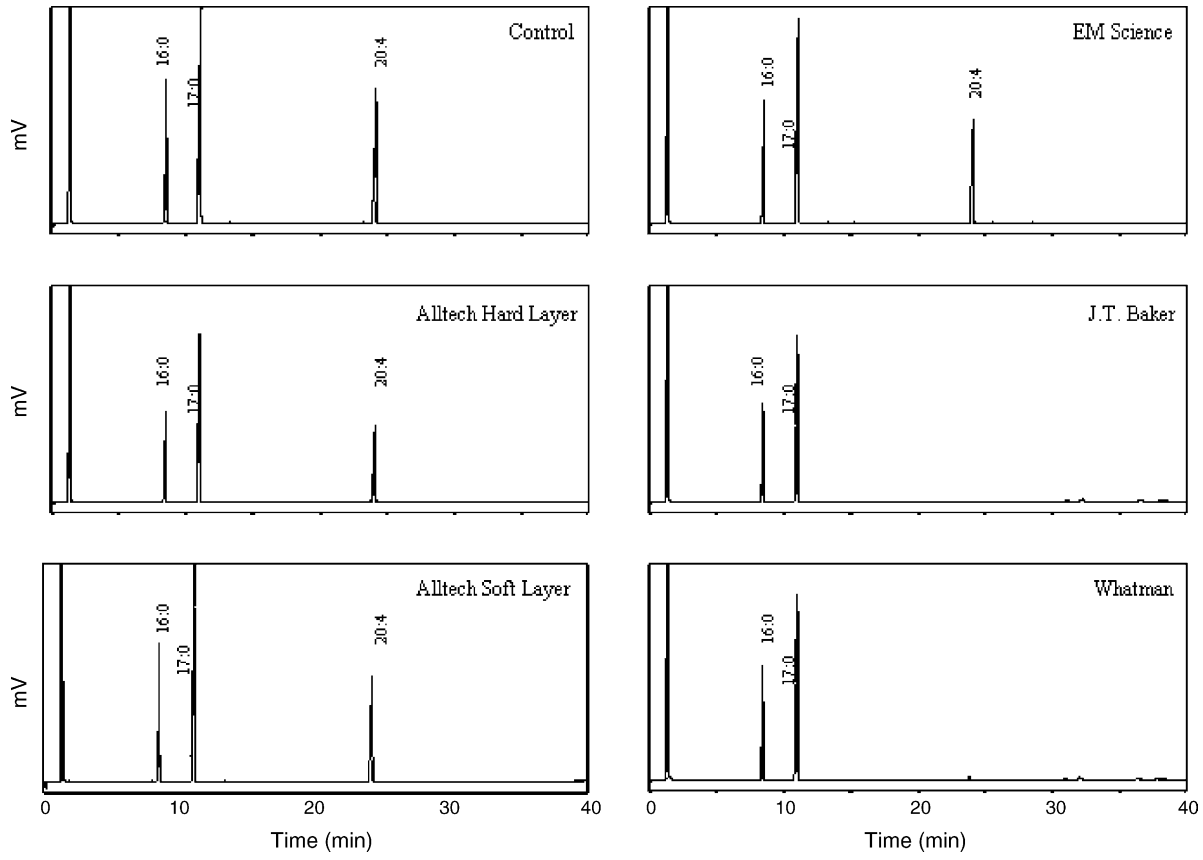

Fig. 3. Gas chromatograms of methyl esters of 16:0–20:4 PC and 17:0–17:0 PC after the chromatography of PC on various TLC plates. The quantitative recoveries of 16:0 and 20:4 were calculated relative to those in control sample, and are shown in Table 1.

PC samples (16:0–18:1 and 16:0–18:2) in the presence of loose silica gel (80 mg, corresponding to the size of the PC band) scraped from the various commercial plates. The plates were activated at 110 °C for 30 min. and cooled to room temperature, before scraping the blank silica gel from the area equivalent to the PC spot. Methylation was carried out with BF_3 in methanol in presence of the internal standard 17:0–17:0 PC. As shown in Table 3, both 18:1 and 18:2 were lost in presence of silica gel scraped from J.T. Baker and Whatman plates. No loss occurred in presence of the silica gel scraped from the other three brands. This shows that the silica gel from J.T. Baker and Whatman plates contains an interfering substance that specifically results in the loss of unsaturated fatty acid during the methylation step.

3.4. Effect of methylation conditions

In all the above studies the methylation of the fatty acids was carried out using BF_3 in methanol. Since this procedure involves heating the samples at 90 °C for 1 h, it is possible that the oxidation of unsaturated fatty acid occurred during this step, although the reaction was carried out under N_2. To address this, we methylated 16:0–18:1 PC and 16:0–18:2 PC by acetyl chloride at room temperature, after their chro-

Table 2
Percent recovery of fatty acids from CE and FFA after separation on different brands of TLC plates

	Control (no TLC)	Alltech Hard	Alltech Soft	EM Science	J.T. Baker	Whatman
18:1 CE	100.0	102.0	104.8	106.2	5.7	4.1
18:1 FFA	100.0	102.2	96.4	97.8	0.8	0.9
18:2 CE	100.0	106.1	112.2	120.3	19.6	12.4
18:2 FFA	100.0	88.0	93.0	90.9	1.4	0.8
20:4 CE	100.0	94.6	94.8	104.0	45.5	31.8
20:4 FFA	100.0	86.0	98.1	86.9	0.0	0.0

The CE and FFA samples were first run on the different brand TLC plates, and then methylated with BF_3–methanol. The recovery from GC column was calculated using the internal standard of 17:0 CE or 17:0 FFA. The recovery in control sample (no TLC) was taken as 100%, and all other recoveries were expressed as percent of this value. Values shown are averages of two separate analyses, which differed by <10% from each other.

Table 3
Recovery of PC fatty acids after methylation in presence of silica gel scrapings from various TLC plates

	Control (no silica gel)	Alltech Hard	Alltech Soft	EM Science	J.T. Baker	Whatman
16:0–18:1 PC						
16:0	100.00	100.00	101.20	98.50	98.80	98.90
18:1	100.00	100.00	105.40	104.90	0.70	4.90
Degradation products	0.00	0.77	2.04	5.76	11.75	14.55
16:0–18:2 PC						
16:0	100.00	100.01	101.89	101.30	101.90	104.40
18:2	100.00	101.89	100.29	105.20	3.10	5.40
Degradation products	0.00	0.00	0.00	5.16	23.59	41.02

Silica gel (80 mg) was scraped from the indicated TLC plate and added to 0.1 umol of PC samples (plus 0.1 μmol of di-17:0 PC, the internal standard) and the samples were methylated with BF_3–methanol. The recovery of each fatty acid was calculated relative to the recovery in control (methylation without silica gel) sample.

Table 4
Percent recovery of PC fatty acids after methylation with acetyl chloride

	Control (no TLC)	Alltech Hard	Alltech Soft	EM Science	J.T. Baker	Whatman
16:0–18:1 PC						
16:0	100.00	117.60	124.30	116.90	117.70	117.40
18:1	100.00	82.50	82.90	80.60	1.40	0.00
Degradation products	0.00	1.90	1.70	6.00	120.60	123.30
16:0–18:2 PC						
16:0	100.00	107.47	107.25	108.31	108.83	108.51
18:2	100.00	81.79	84.30	86.13	0.00	0.00
Degradation products	0.00	1.07	0.00	7.62	84.04	65.82

The PC samples were first run on the various TLC plates, and PC spots were methylated with acetyl chloride, instead of BF_3–methanol. The recoveries were calculated as described in the text, and are averages of two separate experiments.

matography on plates from the various brands of silica gel. As shown in Table 4, the loss of 18:1 and 18:2 occurred again only with J.T. Baker and Whatman plates, showing that the high temperature and the methylation reagent used were not responsible for the differential recoveries of unsaturated fatty acids from the various silica gels.

3.5. Recovery of methyl esters of fatty acids

To further differentiate between the loss of fatty acids during the methylation step versus TLC separation step, we first methylated 16:0–18:2 PC or 16:0–18:1 PC in presence of equimolar amounts of 17:0–17:0 PC, and separated the methyl esters on the various plates with the solvent system of hexane:diethyl ether:acetic acid (70:30:1, v/v/v). The methyl ester spots were eluted with diethyl ether, concentrated, and directly injected into GC, without further treatment. As shown in Table 5, there was no loss of the unsaturated fatty acid on any of the plates when the methylation step was avoided after TLC. These results suggest that the degradation of the unsaturated fatty acids observed with the J.T. Baker and Whatman plates occurred only if the silica gel is present during the methylation step. It is possible that an unknown component in these two silica gel preparations either interferes specifically with the methylation of unsaturated fatty acids, or promotes their oxidation during methylation. It is also possible that the methyl esters of FA are more stable compared to the esters of glycerol or cholesterol.

Table 5
Recovery of fatty acid methyl esters after separation on various TLC plates

	Control	Alltech Hard	Alltech Soft	EM Science	J.T. Baker	Whatman
16:0–18:1 PC						
16:0	100.00	103.99	100.31	94.48	99.75	98.26
18:1	100.00	96.29	97.76	99.90	98.54	100.61
16:0–18:2 PC						
16:0	100.00	99.72	101.66	99.24	102.20	98.71
18:2	100.00	98.28	95.67	98.05	115.80	99.19

The PC samples were first methylated with BF_3–methanol, and the fatty acid methyl esters were then run on the various TLC plates with the solvent system of hexane:diethyl ether:acetic acid (70:30:1, v/v/v). The spots corresponding to FAME standard were scraped, eluted with diethyl ether, and analyzed on GC. The control samples were not run on TLC prior to analysis.

Table 6
Recovery of PC fatty acids after elution of PC from the plate before methylation

	Control (no TLC)	Alltech Hard	Alltech Soft	EM Science	J.T. Baker	Whatman
16:0–18:1 PC						
16:0	100.00	103.12	127.30	123.57	131.61	134.01
18:1	100.00	94.37	113.76	115.56	106.70	107.67
16:0–18:2 PC						
16:0	100.00	100.27	109.81	105.04	103.05	102.42
18:2	100.00	94.89	99.09	96.99	89.07	91.31

The PC samples were separated on the indicated TLC plate, and the spots eluted from the plates by the Bligh and Dyer procedure [11]. The eluted samples were then methylated with BF_3–methanol, and analyzed on GC; 17:0–17:0 PC was used as internal standard in all samples for calculation of recoveries. The values shown are averages of two separate analyses.

3.6. Methylation of eluted samples

PC samples (16:0–18:2 and 16:0–18:1) were first chromatographed on all brands of commercial plates as described above, and the PC spots were eluted from the silica gel by extracting two times with 1.9 ml of Bligh and Dyer [11] solvent (water:methanol:chloroform, 0.4:1.0:0.5, v/v/v). To the combined extracts (3.8 ml) 1.0 ml of choloroform and 1.0 ml of water was added, and the chloroform layer recovered by centrifugation. The chloroform was then dried and the lipid methylated with BF_3–methanol as described above, and run on GC. As shown in Table 6, there was no significant loss of unsaturated fatty acid from PC samples run on any plate by this procedure. This shows that the presence of silica gel is necessary during the methylation step for the loss of unsaturated fatty acids to occur.

3.7. Recovery of fatty acids from natural PC

The effect of TLC separation on commercial plates was then tested on the recovery of different unsaturated fatty acids present in PC of human plasma. Total lipids were extracted [11] from a 200 μl sample of normal human plasma after adding the internal standard, 17:0–17:0 PC (0.1 μmol), and separated on various TLC plates, with the solvent system of chloroform:methanol:water (65:25:4, v/v/v). After exposing the standard lane to the iodine vapors, the spot corresponding to PC in the sample was scraped and methylated with BF_3–methanol, and was analyzed by GC. The major unsaturated fatty acids in plasma PC are 18:2, 18:1, 20:3, 20:4, and 22:6 [7,12,13,10,9]. As shown in Table 7, the recovery of the unsaturated fatty acid was in the expected range of literature values, when plates from Alltech and EM Science were used. There were, however, significant losses of all unsaturated fatty acids when plates from J.T. Baker or Whatman were used. On average, there was a 40–50% loss of the unsaturated fatty acids of PC (in terms of μg/ml plasma) on the J.T. Baker and Whatman plates, compared to the other three plates. There was only a minimal loss in 16:0 and 18:0, showing that the effect is specifically on the unsaturated fatty acids. It is of interest to note that the loss of unsaturated fatty acids from plasma PC was significantly less than when individual synthetic PCs were analyzed using the same plates (Figs. 1–3, Table 1). It is possible that the ratio of silica gel to the unsaturated fatty acid is much lower in the case of plasma PC sample, or that the presence of other fatty acids protects the fatty acids from oxidation.

In summary, the results presented here show that some commercial TLC plates are not suitable for the direct methylation of fatty acids in presence of the silica gel, after the TLC separation of the parent lipid classes. There is a selective loss of unsaturated fatty acids in presence of J.T. Baker

Table 7
Recovery of fatty acids from plasma PC after separation on various silica gel plates

FA	Alltech Hard		Alltech Soft		EM Science		J.T. Baker		Whatman	
	% of total	μg/ml	% of total	μg/ml	% of total	μg/ml	% of total	μg/ml	% of total	μg/ml
16:0	24.76	325.30	24.92	381.56	24.75	356.76	28.97	344.67	27.68	330.39
16:1	0.95	12.46	1.00	15.34	0.96	13.88	0.88	10.46	0.79	9.49
18:0	15.59	204.95	14.42	220.71	14.72	212.21	17.41	207.24	16.99	202.96
18:1 ($n-9$)	10.82	142.19	10.72	164.06	9.79	141.18	7.20	86.25	6.26	74.92
18:1 ($n-7$)	1.06	13.92	1.08	16.56	0.93	6.74	0.89	5.53	0.88	5.37
18:2	22.37	295.30	24.03	368.33	23.87	344.05	17.22	205.16	15.32	182.86
20:3	2.03	26.91	2.25	34.45	2.28	32.83	1.62	19.27	1.36	16.23
20:4	9.97	132.12	11.33	173.77	11.76	169.42	8.09	96.55	6.82	81.35
22:6	1.02	13.49	1.59	24.43	1.41	20.27	0.80	9.48	0.67	8.01

Total lipids were extracted from aliquots of normal human plasma, and chromatographed on various TLC plates in chloroform:methanol:water (65:25:4, v/v/v) after adding di-17:0 PC as the internal standard. The spot corresponding to PC was then scraped, methylated with BF_3–methanol, and analyzed on GC. The values shown are averages of duplicate determinations of the same plasma sample.

and Whatman plates, apparently due to oxidative degradation that occurs during the methylation step. No loss of unsaturated fatty acids occurs even on these plates if the lipids are first eluted from the silica gel. Therefore these plates can be used for fatty acid analysis only if the lipids are first eluted before methylation. On the other hand, the Alltech and Merck plates are suitable for direct methylation also. We have not been able to identify the exact nature of the interfering substance in the J.T. Baker and Whatman plates because the exact composition of these plates was not available to us, due to proprietary considerations. We have obtained samples of the polymer and silica gel used in the preparation of Whatman plates, and tested their effect on the recovery of 16:0–18:2 PC. When the PC was methylated in presence of silica gel with or without 2.5% polymer, there was no loss of 18:2 in the resulting methyl esters, suggesting that neither the silica gel nor the polymer may be directly responsible for the loss (results not shown). It is possible that other proprietary substances used in the preparation of these plates or the processing of the plates during their manufacture caused the artifacts. It should be pointed out that we have used the Whatman brand plates (purchased from Fisher Scientific) in many of our past studies [14,15] without any loss of unsaturated fatty acids. Therefore, the recent batches of plates are apparently different from the previous batches we used from the same company.

References

[1] T.L. Sestak, N. Alavi, P.V. Subbaiah, Kidney Int. 36 (1989) 240.
[2] J.T. Lane, P.V. Subbaiah, M.E. Otto, J.D. Bagdade, J. Lab. Clin. Med. 118 (1991) 120.
[3] P.V. Subbaiah, R.A. Rodby, Metabolism 43 (1994) 1126.
[4] P.V. Subbaiah, C.H. Chen, J.D. Bagdade, J.J. Albers, J. Biol. Chem. 260 (1985) 5308.
[5] B. Banerji, P.V. Subbaiah, R.E. Gregg, J.D. Bagdade, J. Lipid Res. 30 (1989) 1907.
[6] P.V. Subbaiah, P.H. Pritchard, Biochim. Biophys. Acta 1003 (1989) 145.
[7] P.V. Subbaiah, D. Kaufman, J.D. Bagdade, Am. J. Clin. Nutr. 58 (1993) 360.
[8] A. Kuksis, in: A. Kuksis (Ed.), Fatty Acid Composition of Glycerolipids of Animal Tissues, vol. 1, New York, 1978, pp. 381–442.
[9] A. Ohta, M.C. Mayo, N. Kramer, W.E. Lands, Lipids 25 (1990) 742.
[10] W. Sattler, H. Reicher, P. Ramos, U. Panzenboeck, M. Hayn, H. Esterbauer, E. Malle, G.M. Kostner, Lipids 31 (1303) (1996) 9.
[11] E.G. Bligh, W.J. Dyer, Can. J. Biochem. Physiol. 37 (1959) 911.
[12] G.B. Phillips, J.T. Dodge, J. Lipid Res. 8 (1967) 676.
[13] B.J. Holub, D.J. Bakker, C.M. Skeaff, Atherosclerosis 66 (1987) 11.
[14] L. Gesquiere, W. Cho, P.V. Subbaiah, Biochemistry 41 (2002) 4911.
[15] R.M. Sargis, P.V. Subbaiah, Biochemistry 42 (2003) 11533.