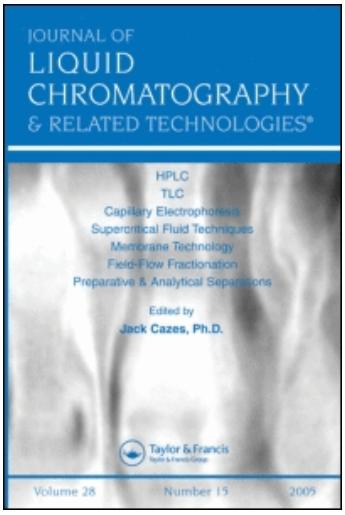

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparison of Reagents for Lipid and Phospholipid Detection and Densitometric Quantitation on Silica Gel and C_{18} Reversed Phase Thin Layers

Joseph Sherma[a]; Susan Bennett[a]
[a] Department of Chemistry, Lafayette College Easton, PA

To cite this Article Sherma, Joseph and Bennett, Susan(1983) 'Comparison of Reagents for Lipid and Phospholipid Detection and Densitometric Quantitation on Silica Gel and C_{18} Reversed Phase Thin Layers', Journal of Liquid Chromatography & Related Technologies, 6: 7, 1193 — 1211
To link to this Article: DOI: 10.1080/01483918308079991
URL: http://dx.doi.org/10.1080/01483918308079991

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(7), 1193-1211 (1983)

COMPARISON OF REAGENTS FOR LIPID AND PHOSPHOLIPID DETECTION AND DENSITOMETRIC QUANTITATION ON SILICA GEL AND C_{18} REVERSED PHASE THIN LAYERS

Joseph Sherma and Susan Bennett
Department of Chemistry
Lafayette College
Easton, PA 18042

ABSTRACT

A variety of chromogenic and fluorogenic detection reagents were evaluated for detection and densitometric quantification of lipids and phospholipids on silica gel and chemically bonded octadecylsilane reversed phase thin layer plates. Phosphosphomolybdic acid (PMA) was found to be the most generally favorable reagent, followed by cupric acetate and ethanolic sulfuric acid. The quantification of lipids in blood serum by chemically bonded RP-TLC was demonstrated using PMA with direct sample application to preadsorbent plates.

INTRODUCTION

The determination of neutral lipids and phospholipids in various biological samples is one of the areas of greatest application for qualitative and quantitative thin layer chromatography (1). A great number of detection reagents have been used for these determinations, but only a few limited comparisons have been reported (e.g., 2,3). This paper reports a comparative study of the suitability of common chromogenic and fluorogenic reagents for detection and quantification by scanning of lipids and phospholipids on silica gel and C_{18} chemically bonded reversed phase silica gel thin

 0148-3919/83/0607-1193$3.50/0

layer plates. Reagents were evaluated on the basis of convenience, visual detection sensitivity, stability of zones, densitometric sensitivity, reproducibility of scanning, and the nature of the calibration curve. Detection and quantification on silica gel and reversed phase layers were also compared.

Phosphomolybdic acid (PMA) and cupric acetate proved to be the most advantageous reagents, and PMA was especially useful for quantifications on C_{18} layers. This was demonstrated by determination of cholesterol, triolein, and cholesteryl oleate in human blood serum by reversed phase TLC with densitometry.

EXPERIMENTAL

Materials

Lipid standards were purchased from Nu-Chek-Prep, Inc. and phospholipids from Avanti Polar Lipids, Inc. The lipids studied were methyl oleate, cholesteryl oleate, triolein, and cholesterol. The phospholipids were lecithin (dipalmitoyl); lecithin (egg); sphingomyelin (bovine); and phosphatidyl ethanolamine (egg), inositol (bovine), serine (bovine), and glycerol (egg). Stock solutions of each compound were prepared at a concentration of 1.00 µg/µl, and volumetric dilutions were made to give solutions containing 100, 50.0, 10.0, and 1.00 ng/µl. Lipids were dissolved in chloroform, and phospholipids in chloroform-methanol (1:1) except for phosphatidyl inositol (hexane-ethanol, 9:1) and phosphatidyl serine (benzene).

Whatman 20 x 20 cm scored, preadsorbent silica gel plates (LK5DF), 10 x 20 cm scored, preadsorbent high performance silica gel plates (LHPKDF), and 20 x 20 cm scored, preadsorbent C_{18} chemically bonded reversed

phase plates ($LKC_{18}DF$) were used. Comparable plates without fluorescent indicator were used for reagents producing spots fluorescing at 254 nm. To remove impurities and increase sensitivity of detection reagents, plates were prewashed by development with chloroform-methanol (1:1) (silica gel) or chloroform-acetonitrile (80:20)(C_{18}) and dried in a fume hood prior to spotting. The properties of these plates and general techniques for their proper utilization have been described (4).

Procedures

Solutions were applied to the preadsorbent spotting area by streaking with a 25 µl Drummond Dialamatic microdispenser, followed by drying with warm air from a hair drier. Plates were developed in filter paper-lined glass chambers that were equilibrated with the mobile phase for approximately 15 minutes. One hundred ml of solvent gave a pool in the chamber that was about 3 mm deep. Silica gel TLC and C_{18} layers were developed for 10-12 cm and HP silica gel 6 cm.

Developed plates were air dried in a fume hood, and detection reagents were applied either by spraying from a Kontes Chromaflex sprayer or by dipping in a Thomas-Mitchell metal dip tank as described in the individual sections below. Heating steps were carried out in a temperature-regulated Precision Model 15 or Blue M Electric Co. Model OV-8A oven.

Zones were scanned with a Kontes Model 800 fiber optics densitometer equipped with a Hewlett Packard Model 3390A calculating integrator/recorder. Scanning was done in the double beam mode using the source phosphor found to be most appropriate, based on maximum signal-to-noise ratio, for each detection reagent. A shroud was used to concentrate the light source. Cali-

bration curves were plots of integrator areas vs nanograms spotted for a series of standard spots.

Fingertip blood serum was analyzed on C_{18} plates by the following procedure: the first few drops from a pricked finger were rejected, and the sample (about 5 µl) was then allowed to flow freely into tubes designed for use with a Fisher Model 59 centrifuge. Blood was centrifuged at 7000 rpm for 5 minutes, and the serum was diluted 1:10 or 1:20 with distilled water to produce a spot with an area falling on the linear portion of the calibration curve for the compound of interest when 5 µl was applied for TLC. The initial zones of the sample (spotted in duplicate) and bracketing standards were dried for 5 min with a hair drier, and the plate was developed with acetonitrile-methyl ethyl ketone-chloroform-acetic acid (50:30:15:5) for a distance of 13 cm (about 25 minutes). Chromatograms were oven dried (5 min, 100°C), cooled, dipped in Whatman 5% PMA reagent, and heated for 8 minutes at 110-120°C. The plates were cooled and scanned, and the average sample spot area was interpolated from the calibration curve constructed from the standards in the same plate to give the ng of lipid present. Conversion was made to mg/100 ml present in blood based on the amount of sample spotted and the dilution factor.

RESULTS AND DISCUSSION

Lipid and phospholipid visualization reagents were evaluated on silica gel TLC and HPTLC layers and on C_{18} RP layers in terms of ability to detect 2-3 µg amounts statically (without development); visual and scanning sensitivity after development; densitometric calibration curve (slope, linearity); stability of detection (by scanning a single zone repeatedly over a

period of time); and quantification reproducibility (relative standard deviation of the scan areas of six standard zones after spotting, development, and detection). Mobile phases used on silica gel for the calibration curves, reproducibility, and time studies were chloroform-ethyl acetate (94:6) for cholesterol (R_F 0.40), hexane-ethyl ether-acetic acid (80:20:1) for methyl oleate (R_F 0.60), and chloroform-ethyl acetate-n-propanol-methanol-0.25% aq. KCl (25:25:25:13:9) for sphingomyelin (R_F 0.10) and lecithin (R_F 0.20). On HP silica gel, chloroform-methanol-ethanol-acetic acid (40:30:20:10) or chloroform-methanol-ethanol-0.25% KCl (40:20:20:20) were used to provide increased R_F values for lecithin and sphingomyelin. On C_{18} RP layers, ethanol-acetic acid-formic acid (80:10:10) was used for cholesterol (R_F 0.35), acetonitrile-chloroform (60:40) for cholesteryl oleate (R_F 0.30) and triolein (R_F 0.30), and acetonitrile-methyl ethyl ketone-chloroform (50:35:15) for methyl oleate (R_F 0.55). These mobile phases were designed to yield R_F values that were usually within the optimal range of 0.3-0.7 for densitometric analysis (5). On HP plates, the short (6 cm) development distance required an R_F value of at least 0.25 so that the spot could be scanned without interference from the preadsorbent-sorbent junction.

The Nu-Chek Prep 18-4A neutral lipid standard mixture was completely separated on a reversed phase layer using the mobile phase acetonetrile-methyl ethyl ketone-chloroform-acetic acid (50:35:15:5). R_F range values were cholesteryl oleate 0.19-0.22, triolein 0.25-0.30, cholesterol 0.31-0.36, methyl oleate 0.58-0.60, and oleic acid 0.65-0.67 after a 13 cm development (25 minutes). The R_F values for this mixture on silica gel developed with hexane-diethyl ether-acetic acid (80:20:1) were

cholesterol 0.08, oleic acid 0.24, triolein 0.37, methyl oleate 0.49, and cholesteryl oleate 0.62. Comparisons of these values indicate some sequence changes but not the exact reversal that would be expected if the mechanism on the C_{18} layer was totally reversed phase partition. Phospholipids were difficult to quantify on C_{18} layers because four of the six natural standards gave two zones upon development with mobile phases such as those listed above and detection with PMA. For example, development of phospholipid standards on C_{18} with ethanol-acetic acid-H_2O (80:10:10) gave the following R_F values: (lecithin 0.21-0.27 Ostreak), phosphatidyl serine 0.39 + 0.45, sphingomyelin 0.17 + 0.26, and phosphatidyl glycerol 0.47 + 0.51. These multiple compounds apparently were not resolved on silica gel in the mobile phases employed in this research. Phosphatidyl ethanolamine and phosphatidyl inositol gave single spots with respective R_F values of 0.46 and 0.49 in this mobile phase.

The following subsections describe procedures and results for the various detection reagens. In each case all four lipids and seven phospholipids were tested for static detection, and selected compounds were developed and evaluated in terms of sensitivity, stability, and densitometry.

Phosphomolybdic Acid (PMA)

PMA was prepared as a 5% solution in absolute ethanol, followed by vacuum filtration through a Buchner funnel. The solution is bright yellow when fresh but darkens to yellow-green on storage. Whatman PMA reagent (Catalog No. 4911-119) was found to be equivalent to fresh laboratory-prepared solution and not to require storage in a refrigerator. Chromatograms were dipped

into the reagent and then heated at 110-120°C for 8-12 minutes to produce blue spots on a yellow background. Overheating caused darkening of the background. PMA can be incorporated into the layer by dipping prior to development if the mobile phase will not wash out the reagent (6). Pre-dipping was possible with the phases used for lipids but not those for the phospholipids. Since there were no significant advantages found for pre-dipping, post-dipping was routinely used. The dip solution was reused as long as it retained its light yellow color. The red Kontes phosphor was employed to scan spots produced by PMA.

All of the lipids and phospholipids except dipalmitoyl lecithin (containing only saturated phospholipid) were strongly detected at 2 μg level statically on both silica gel TLC and HPTLC layers. The visual and scanning detection limits were 100 and 200 ng, respectively, for silica gel TLC and 50 ng and 100 ng for HPTLC. Scan areas were stable for at least 60 minutes. On silica gel, calibration curves were typically linear from 200-1200 ng with correlation (linearity) coefficients (r) of 0.98. Reproducibility of scanning six spots ranged from 3.1-4.9% for both lipids and phospholipids. On HP silica gel, linearity was obtained from 100-1000 ng, r was approximately 0.96, and reproducibility was 1.4-4.8%. Figure 1 shows a calibration curve for sphingomyelin on HP silica gel.

On C_{18} RP layers, all compounds were detected with visual limits of 25-100 ng and scanning limits of 50-200 ng. Calibration curves were linear to about 1000 ng and r values were at least 0.98. Reproducibility of quantification for cholesterol was 4.9%. A calibration curve for cholesteryl oleate is illustrated in Figure 2.

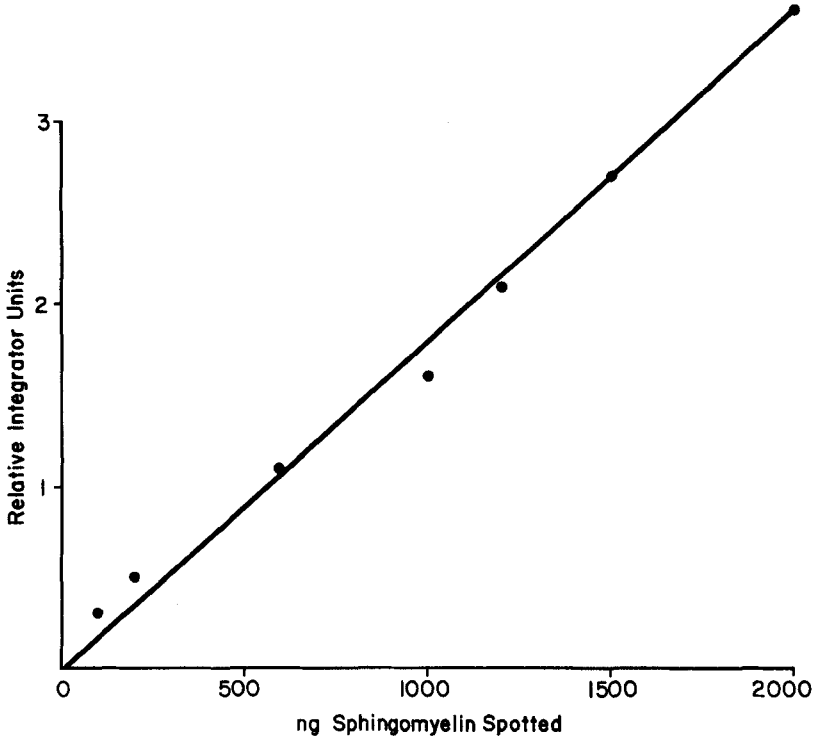

Figure 1. Calibration curve for 100-2000 ng of sphingomyelin developed on a high performance silica gel plate with chloroform-methanol-ethanol-0.25% KCl (40:30:20:10) and detected with PMA.

Scans of the peaks used to construct this curve are shown in Figure 3.

Cleaning of plates by predevelopment was very important in obtaining a light yellow background with PMA, and use of an oven with even heat distribution was important for good precision. Freshness of the PMA solution was more important on RP plates than on silica gel. Laboratory-made solution could be used only within 24

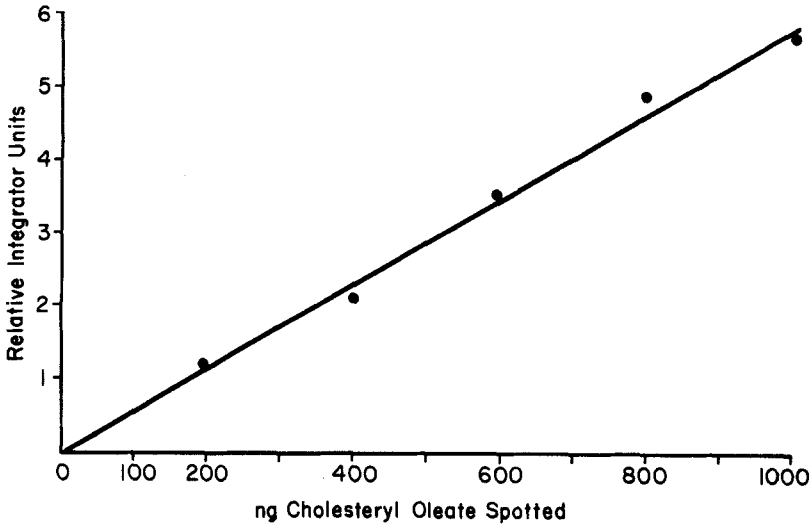

Figure 2. Calibration curve for 200 to 1000 ng of cholesteryl oleate developed on a C_{18} reversed phase plate with acetonitrile-chloroform (60:40) and detected with PMA.

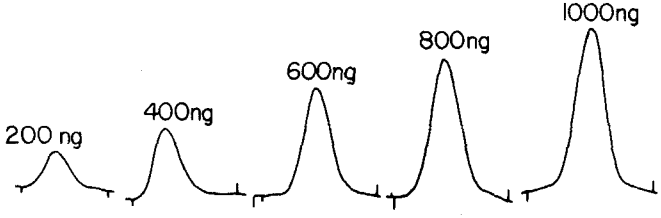

Figure 3. Scans of 200-1000 ng of cholesteryl oleate on a C_{18} layer using the Kontes Model 800 scanner and Hewlett Packard Model 3390A integrator with attenuation X8.

hours of preparation. However, Whatman PMA reagent solution was useful over a period of at least one week.

Cupric Acetate

The reagent was prepared by mixing for 1 hour with a magnetic stirrer 30 g of cupric acetate monohydrate and 80 ml of phosphoric acid with sufficient distilled water to make one liter of solution. Plates were dipped into the reagent and then heated (160°C for 8-15 min for silica gel and 120°C for 4-5 minutes for C_{18}) to produce charred spots on a white background. The white phosphor was inserted when scanning the zones (7).

All compounds were statically detected at the 2 µg level on silica gel except dipalmitoyl lecithin (saturated) (8). Visual and scanning sensitivity were both 100 ng for cholesterol and sphingomyelin and 200 ng/400 ng for lecithin. The linear range of calibration curves was typically 100-1000 ng and correlation coefficients were 0.96-0.99. The reproducibility for cholesterol was 4.4% and for lecithin 7.1%. A time study indicated stability within 2.5% over a period of 30 minutes upon scanning of a 400 ng cholesterol zone.

Visual detection limits on HP silica gel were 25 ng for cholesterol and lecithin, and 50 and 100 ng for these compounds, respectively, for scanning. Calibration curves were linear up to 1000-1200 ng, with r values of 0.91-0.98. Reproducibility for cholesterol and lecithin were 2.9 and 4.3%, respectively.

Cupric acetate was not successfully applied to C_{18} layers. The heating time was very critical, and it was virtually impossible to produce dark spots with good contrast before the layer itself began to char. Even on silica gel, the time and temperature of heating were

more critical for reproducible zone color and a light, consistent background than with PMA. Zone scans, baselines, calibration curves, and r values also tended to be less erratic for PMA compared to cupric acetate. The best results that could be achieved on RP layers were a 200 ng visible detection limit for cholesterol and a 16% RSD reproducibility for scanning replicate zones.

Cupric Sulfate

The reagent was prepared by dissolving 100 g of anhydrous cupric sulfate plus 80 ml of phosphoric acid in enough water to make one liter and magnetically stirring for 1 hour(8). Plates were dipped and heated for 10-15 minutes at 160°C to produce charred zones on a white background. The white phosphor was used to scan these zones.

Static detection of all compounds, including dipalmitoyl lecithin (saturated), was obtained at the 2 µg level. The detection of saturated phospholipid with $CuSO_4$ reagent but not cupric acetate has already been reported (8).

Detection limits of lipids and phospholipids were 50-100 ng visually and 100-200 ng for scanning on silica gel. Limits for dipalmitoyl lecithin were 200 and 400 ng, respectively. Calibration curves were linear to 1200 ng, with r values of 0.90-0.98. RSD values were 2.5% for cholesterol and 1.5% for egg lecithin. On HP silica gel, detection levels were similar, but calibration curves had considerably lower r values due to zone scans with uneven baselines that were difficult to measure accurately. Backgrounds were generally darker and less even than with cupric acetate reagent. Two spots were detected for some of the natural phospholipids which

had given only one spot with cupric acetate or PMA, indicating the presence of saturated phospholipid in these standards (8). One 800 ng zone of cholesterol was scanned over a 30 min period and areas were constant within 5.5%. Cupric sulfate was not applicable with C_{18} layers; the plate turned dark before spots appeared with all heating conditions attempted.

Dittmer-Lester Reagent

The reagent was prepared from molybdic anhydride and molybdenum in acid solution as described earlier (4,10). The plate was sprayed uniformly until lightly damp to produce blue zones for phospholipids, but lipids were not detected. Visual detection limits were about 1 µg for dipalmitoyl and egg lecithin and phosphatidyl ethanolamine, and approximately 2 µg for the others after development. The blue spots appeared on a lighter blue background and were blotchy and grainy; after 4 hours, the spots became white. The blue zones were not suitable for scanning on either silica gel TLC or HPTLC plates. On C_{18} layers, only phosphatidyl serine and glycerol were detected at the 2 µg level during static tests, and these spots were blotchy and unstable.

Phospray

The prepared reagent from Supelco was applied by using a procedure recommended by the manufacturer during a phone conversation. The chromatogram was thoroughly air dried, heated at 100°C for 10 sec, sprayed moderately, and then re-heated for 30-60 seconds. Green spots were produced against a dark blue background for all phospholipids at the 2 µg level in static tests, with the egg lecithin and phosphatidyl serine and inositol zones being most strongly colored. Lipids were not detected. The plate background was always uneven except after the lightest spraying, which did not allow the phospholipids

to be detected. Whatman K4 gypsum-bound silica gel plates gave much better results than on the originally used K5 silica gel TLC and HPTLC plates, but silica gel G was not included as part of this research project. No detection was obtained for any of the phospholipids or lipids in static tests at the 2-3 µg level on C_{18} RP layers.

Bromothymol Blue

Commercial reagent solution (Supelco No. 3-4656) was sprayed on the plate until wet. All lipids and phospholipids, including depalmitoyl lecithin, were detected on silica gel as aquamarine spots on a green background. Scanning was attempted using the red phosphor, but uneven backgrounds and white shadows near the spots precluded satisfactory results. For example, a reproducibility study with egg lecithin resulted in a 21% relative standard deviation. Sensitivity of visual detection was approximately 50-100 ng. Detection was not successful for lipids or phospholipids on C_{18} RP layers because the layer background was blue.

1,2-Naphthoquinone-4-Sulfonic Acid

The reagent solution (100 mg of 1,2-naphthoquinone-4-sulfonic acid dissolved in 100 ml of a mixture of ethanol-60% $HClO_4$-H_2O-40% formaldehyde, 20:10:9:1) was applied by dipping the plates, which were then heated for 7-8 minutes at 80°C to produce blue spots against a tan background. The red phosphor was used for densitometry. All compounds except dipalmitoyl lecithin were detected on silica gel at minimum levels of 200 ng (visual) to 400 ng (scanning). The calibration curve for cholesterol was linear from 400-1000 ng, the correlation coefficient was 0.99, and reproducibility was 13% (RSD). The major problems with the reagent were the sensitive nature of the heating step required to achieve the maxi-

mum contrast between the spots and the background, as well as the generally blotchy and uneven background produced. These problems were even worse on HP silica gel than on K5 silica gel. On C_{18} RP layers, all compounds were detected at the 200-400 ng level, but dipping the reagent caused the layer to loosen and sometimes come off the glass, precluding use of the reagent for quantification.

Acid Fuchsin-Uranyl Acetate

Attempts to apply this reagent, previously described by Michalec and Reinisova (10) and Michalec and Kolman (11), were unsuccessful. All lipids and phospholipids were detected as faint red spots against a light red, uneven background at the 500-1000 ng level on silica gel. Attempts to dip C_{18} layers caused the layer to be removed from the glass, and spraying the reagents gave poor detection sensitivity and an uneven, colored background. No conditions could be found using these two solutions to obtain red spots on a white background as reported earlier.

6-p-Toluidino-2-Naphthalenesulfonic Acid, Potassium Salt

A 1 mM TNS solution was prepared in 50 mM Tris-HCl, pH 7.4 and lightly sprayed onto the layer (12). All lipids and phospholipids were detected visually on silica gel at 100-1000 ng levels under shortwave UV light, but the spots faded quickly and could not be reproducibly scanned. The reagent could not be used on C_{18} plates because the layer became fluorescent under shortwave UV light after application.

Primuline

The reagent was prepared by diluting 1 ml of stock solution (0.1 g primuline in 100 ml H_2O) to 100 ml with acetone-H_2O)(4:1) (13). Plates were sprayed until wet

and viewed immediately. All lipids and phospholipids were detected visually at the 1 µg level after development as purple spots against a green background on silica gel when viewed under shortwave UV light. As the plate dried after spraying, the spots faded and sensitivity decreased so that reproducible scanning was not possible. No spots were visible under either longwave or shortwave UV light on C_{18} layers.

Sulfuric Acid in Ethanol

Plates were sprayed lightly but uniformly with absolute ethanol-conc. H_2SO_4 (9:1) and heated at 180°C for 5-10 minutes (14). Tan spots on a purple background were visible under longwave UV light on silica gel for all compounds except dipalmitoyl lecithin, but visual sensitivity was not as good as for PMA. Therefore, quantification by scanning was not studied.

The same compounds were visually detected on C_{18} layers at minimum levels ranging from 400 ng-9 µg. Scanning in the longwave UV region was done by placing the 254 nm filter on top of the 351 nm phosphor and taping a plastic "sunscreen" over the densitometer head to block 360 nm radiation from reaching the detector. The calibration curve for cholesterol was linear from 1-3 µg with a correlation coefficient of 0.99. The curve for methyl oleate was linear from 5-9 µg with a linearity of 0.99; 5 µg was the lowest level that gave a peak for this compound, although the visual detection limit was 400 ng. Peak areas were constant over a period of at least 30 minutes, and reproducibility of scanning was 4.7% (RSD). It is important not to overheat the plate or the spots (and eventually the background) will char instead of becoming fluorescent.

Nitric Acid Vapors

Plates were placed in a closed chamber saturated (for 1 hour) with vapors from conc. HNO_3 contained in a 30 ml beaker (15). After 10 minutes exposure, the plates were heated for 10 minutes at 180°C to produce orange-green spots against a purple background when viewed under longwave UV light. Cholesterol, cholesteryl oleate, sphingomyelin, and phosphatidyl serine, inositol, and ethanolamine were visually detected at levels of 2-5 µg after development on silica gel, but background noise precluded successful quantification by scanning. On a C_{18} layer, only phosphatidyl serine was well detected at approximately 2 µg, but again an uneven background did not allow successful scanning.

2',7'-Dichlorofluorescein

Plates were dipped for 10 seconds in the reagent solution (1.5 mg/100 ml isopropanol), air dried for 15 minutes, and viewed under longwave UV light (16). Spots with sufficient contrast to the background to allow sensitive visual detection or quantification were not produced on any of the layers tested.

Conclusions

Based on the above results, in our hands PMA proved to be the overall best reagent for detection and *in situ* quantification of lipids and phospholipids on all three layers considering the aforementioned criteria. Cupric acetate was also excellent for silica gel but not for C_{18} bonded layers. Ethanolic sulfuric acid was the second best reagent found for the RP layers, but the sensitivity of quantification was not equal to PMA.

It must be stressed that our study was made using closely controlled, standardized procedures, but only with three types of polymer-bound (hard) layers that

are the most widely used today. Results with any given reagent might be different on another type of layer, e.g., silica gel G, which may have been used when the reagent was originally reported in the literature, or if a different scanner was used.

Analysis of Blood Serum

To demonstrate the application of RP layers and PMA for the analysis of lipids in biological fluids, blood serum from a single subject, an apparently healthy adult male, was analyzed by direct spotting on C_{18} preadsorbent plates, as described in the Experimental section, followed by development with acetonitrile-methyl ethyl ketone-chloroform-acetic acid (50:30:15:5). The average values of morning blood were 50.0 mg of cholesterol and 32.0 mg of cholesteryl oleate per 100 ml. Figure 4 shows the scans of duplicate 5 µl of this serum after dilution (1 + 9) with water. The peak areas for cholesterol agreed within 7.7% and for cholesteryl oleate within 1.3%. The triolein content was not quantified. Blood taken from the same subject in the afternoon of the same day had 67.5 mg/100 ml of cholesterol and 36.0 mg/100 ml of cholesteryl oleate.

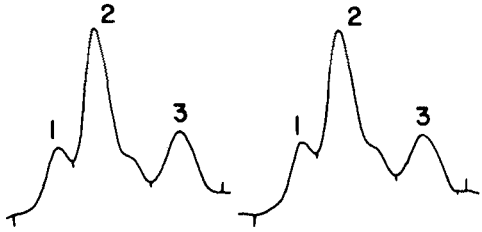

Figure 4. Scans of chromatograms of duplicate 5 µl blood serum samples; peak 1 is cholesterol, 2 is triolein, and 3 is cholesteryl oleate.

On day 2 of the study, morning blood contained 28.0 mg/100 ml of cholesteryl oleate and 69.0 mg of cholesterol. Triolein was again identified but not quantified. On day 3, morning and afternoon blood samples were collected, diluted 20:1, and the triolein content was determined to be 240 mg/100 ml in both samples. In all analyses, duplicate samples agreed within 10% and usually within 5%.

These limited analyses demonstrate the practicality of preadsorbent RP-TLC for lipid determination using PMA detection reagent.

REFERENCES

1. Sherma, J. and Fried, B. Anal. Chem., 54(5), 45R (1982).

2. Bitman, J. and Wood, D. L., J. Liq. Chromatogr., 5(6), 1155 (1982).

3. Caselli, M., Anal. Lett., 14(B20), 1693 (1981).

4. Sherma, J., TLC Technical Series, Volumes 1,2, and 3, Whatman Chemical Separation Inc., 9 Bridewell Pl., Clifton, N. J.

5. Touchstone, J. C., Schwartz, M. F., and Levin, S. S., J. Chromatogr. Sci., 15, 528 (1977).

6. Touchstone, J. C., Balin, A. K., Murawec, T., and Kasparow, M., J. Chromatogr. Sci., 8, 443, (1970).

7. Touchstone, J. C., Hansen, G. R., Zelop, C., and Sherma, J., in Advances in Thin Layer Chromatography: Clinical and Environmental Applications (Touchstone, J. C., ed.), Wiley-Interscience, New York, 1982, Chapter 16, p. 219.

8. Touchstone, J. C., Levin, S. S., Dobbins, M. F., and Carter, P. J., J. High Resolut. Chromatogr. Chromatogr. Commun., 4(8), 423 (1981).

9. Ryu, E. K. and MacCoss, M., J. Lipid Res. 20, 561 (1979).

10. Michalec, C. and Reinisova, J., J. Chromatogr., 162 229 (1979).

11. Michalec, C. and Kolman, Z., J. Chromatogr., 22, 385 (1966).

12. Jones, M., Keenan, R. W., and Horowitz, P., J. Chromatogr., 237, 522 (1982).

13. Wright, R. S., J. Chromatogr., 59, 220 (1971).

14. Touchstone, J. C., Levitt, R. E., Levin, S. S., and Soloway, R. D., Lipids, 15(5), 386 (1980).

15. Zhou, L., Shanfield, H., and Zlatkis, A., J. Chromatogr., 239, 259 (1982).

16. Ng, D. S. and Blass, K. G., J. Chromatogr., 163, 37 (1979).