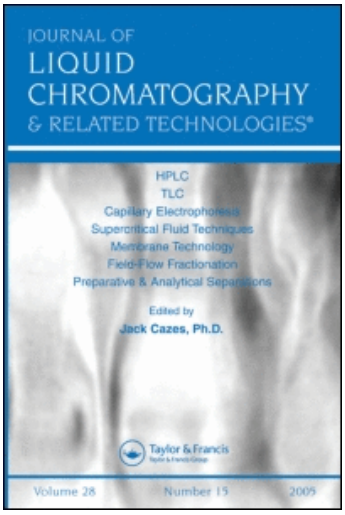

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Neutral Lipids in Regular and Lowfat Eggs by High Performance TLC with Densitometry

M. C. Smith[a]; C. L. Webster[a]; J. Sherma[a]; B. Fried[a]
[a] Department of Chemistry Department of Biology, Lafayette College Easton, Pennsylvania

To cite this Article Smith, M. C. , Webster, C. L. , Sherma, J. and Fried, B.(1995) 'Determination of Neutral Lipids in Regular and Lowfat Eggs by High Performance TLC with Densitometry', Journal of Liquid Chromatography & Related Technologies, 18: 3, 527 — 535
To link to this Article: DOI: 10.1080/10826079508009254
URL: http://dx.doi.org/10.1080/10826079508009254

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(3), 527-535 (1995)

DETERMINATION OF NEUTRAL LIPIDS IN REGULAR AND LOWFAT EGGS BY HIGH PERFORMANCE TLC WITH DENSITOMETRY

MICHAEL C. SMITH[1], COURTENAY L. WEBSTER[1], JOSEPH SHERMA[1], AND BERNARD FRIED[2]
[1]*Department of Chemistry*
[2]*Department of Biology*
Lafayette College
Easton, Pennsylvania 18042

ABSTRACT

A simple quantitative HPTLC method was developed for determination of cholesterol esters, triacylglycerols, free fatty acids, and cholesterol in hens' egg yolk. Lipids were extracted with chloroform-methanol (2:1), the extract was passed through a glass wool column, and the eluate was chromatographed on laned preadsorbent silica gel HPTLC plates with bracketing standards using the Mangold developing solvent for the latter three lipid classes or a modified Mangold solvent for cholesterol esters. Lipids were detected with phosphomolybdic acid spray reagent, and zones were quantified by densitometric scanning. The method was used for the analysis of four regular and four lowfat eggs, and it was found that there was no significant difference between the two types of eggs except for a lower concentration of free fatty acids in the lowfat eggs. There was no significant difference between the mean cholesterol content of either type of eggs in this study and 18 regular eggs analyzed in 1987. Fractions containing monoacylglycerols, diacylglycerols, and nonpolar hydrocarbons were identified in yolk from both types of eggs, but amounts were not quantified.

INTRODUCTION

In an earlier paper (1), a densitometric HPTLC method was developed for determination of cholesterol in egg yolk and was used to survey the cholesterol content of 18 individual eggs. In this study, the HPTLC method was modified by using phosphomolybdic acid (PMA) detection reagent instead of cupric acetate-phosphoric acid reagent for quantification and was extended to the identification and quantification of triacylglycerols, free fatty acids, and cholesterol esters, in addition to cholesterol, in egg yolk. The applicability of the method was demonstrated by performing comparative quantitative analyses of these neutral lipid fractions in regular eggs with no nutritional information listed on the carton compared to eggs purchased in a carton labeled as containing "less than 4% saturated fat" and having a list of nutritional data. It was found that the two types of eggs differed significantly only in the concentration of the free fatty acid fractions.

EXPERIMENTAL

Analyses were performed on Whatman laned preadsorbent high performance silica gel plates using sample preparation (1) and quantitative TLC (2) procedures similar in general to those described earlier. Mixed and individual neutral lipid standards were obtained from Matreya (Pleasant Gap, PA), and standard solutions were prepared in chloroform-methanol (2:1) solvent at a concentration of 0.200 ug/ul of each lipid.

Lipids were extracted from 100 mg egg yolk samples with 2 ml of chloroform-methanol (2:1) in a small vial, and

extracts were passed through a glass wool column held in a disposable pipet. Column effluents were evaporated to dryness under nitrogen and reconstituted in 3.00 ml of chloroform-methanol (2:1) for determination of cholesterol esters and free fatty acids. The reconstituted column effluent was further diluted 1:1 with chloroform-methanol (2:1) for determination of cholesterol (0.25 ml + 0.25 ml of solvent), and a 16-fold dilution of the original reconstituted column effluent was required for determination of triacylglycerols (0.20 ml + 3.00 ml of solvent).

The mobile phases used for development of 10 x 20 cm Whatman (Clifton, NJ) LHP-KDF high performance, preadsorbent silica gel plates in a Camag twin-trough chamber were the Mangold solvent, petroleum ether (37.5-52°C)-diethyl ether-acetic acid (80:20:2) (solvent 1), for determination of cholesterol, triacylglycerols, and free fatty acids, and n-hexane-petroleum ether-ethyl ether-glacial acetic acid (50:20:5:1) (solvent 2) for cholesterol esters. Lipids were detected by spraying the plate with 5% ethanolic PMA and heating at 110-120°C for 5-10 min. A series of sample and standard aliquots between 1.00 and 16.0 ul were applied to each plate using 10 and 25 ul Drummond (Broomall, Pa) digital microdispensers, and quantification was performed by reflectance scanning at 700 nm of the sample and standard zones of each lipid with the closest matching areas using a Shimadzu Model CS-930 densitometer. A response factor was calculated from the area and weight of the scanned standard zone and was used to calculate the weight of lipid in the

sample zone (2). Lipid concentrations are reported in units of mg/g of yolk.

RESULTS AND DISCUSSION

The PMA reagent detected the lipids as flat dark blue bands on a light yellow background. The Mangold solvent (solvent 1) gave excellent resolution of the lipid fractions in standards and samples with the following respective R_f values (Figure 1): cholesterol (sterols), 0.37; oleic acid (free fatty acids), 0.43; triolein (triacylglycerols), 0.74; cholesteryl oleate (cholesterol esters), 0.90. The cholesterol ester zones were located too close to the solvent front to allow their measurement by scanning, so solvent 2, which provided an R_f value of 0.67 (Figure 2), was used for their analysis.

The determination of lipids in the yolks of four regular and four lowfat eggs yielded the values shown in Table 1. It can be seen that the regular eggs had higher concentrations of each of the lipids except cholesterol, for which the regular eggs had a lower value. Student's t-test was used to compare the means between the two types of eggs ($P<0.05$ was considered to indicate a significant difference), and it was found that only the free fatty acid concentrations were significantly different ($P=0.024$). The spread of values found for the concentration of lipids in individual eggs of both types was quite consistent, with coefficient of variation (relative standard deviation) values ranging from 13 to 25% except for free fatty acids in the lowfat eggs (52%).

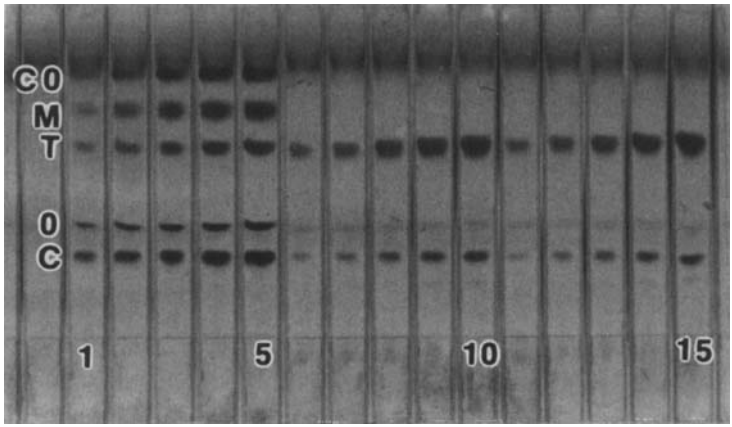

FIGURE 1. Photograph of chromatograms of lipid standards and egg yolk extracts on an HPTLC silica gel plate developed with the Mangold solvent (solvent 1) and detected with PMA reagent. Lanes 1-5 contain 2, 4, 8, 12, and 16 ul aliquots of a solution of Matreya Nonpolar Lipid Mix B having concentrations of 0.2 ug/ul each of cholesteryl oleate (CO), methyl oleate (M), triolein (T), oleic acid (O), and cholesterol (C). Lanes 6-10 and 11-15 contain 2, 4, 8, 12, and 16 ul of two different regular egg yolk extracts diluted for analysis of triacylglycerols.

TABLE 1

LIPID CONTENT (MG/G YOLK) OF REGULAR AND LOWFAT EGGS AS DETERMINED BY THE QUANTITATIVE HPTLC METHOD

Lipid Class	Mean	Range	SD
(Regular Eggs)			
Cholesterol	16.7	14.7-19.6	2.2
Free fatty acids	4.00	2.79-5.10	1.0
Triacylglycerols	216	180-271	43
Cholesterol esters	2.32	1.78-2.89	0.53
(Lowfat Eggs)			
Cholesterol	19.4	14.8-22.3	3.2
Free fatty acids	1.77	0.717-2.94	0.93
Triacylglycerols	190	150-247	42
Cholesterol esters	1.72	1.54-2.06	0.23

SD = standard deviation for 4 replicates in each case

The carton containing the lowfat eggs analyzed in the present study lists a cholesterol content of 390 mg/100 g. This is presumably the average concentration on a whole-egg basis, so it is impossible to directly compare our value of 19.4 mg/g of yolk. The carton also claims that the eggs contain less than 4% saturated fat in the yolk. This value was probably determined by gas chromatographic analysis of the transesterified lipids in whole eggs. We did not examine fatty acid methyl esters and cannot comment on the percentage of saturated fatty acids in our samples. Therefore, our TLC results cannot be compared to the stated value for saturated fat in the lowfat eggs we analyzed.

Analysis of 18 eggs for cholesterol performed in 1987 [1] gave a mean of 15.2 mg/g, a range of 9.72-26.2, and a standard deviation of 4.7. This mean cholesterol concentration is lower than both the current regular or lowfat eggs, but the values are not statistically different based on Student's t-test. It is interesting that any modified feeding protocols instituted by hen breeders over the intervening 7 years to lower cholesterol levels apparently did not have this effect in the samples we analyzed.

The earlier HPTLC method for identification of lipids and quantification of cholesterol in egg yolk (1) was modified by use of the Mangold solvent system (solvent 1) for determination of cholesterol, free fatty acids, and triacylglycerols and a new mobile phase (solvent 2) for cholesterol esters, in place of the Skipski double-development system and chloroform-ethyl acetate (94:6). In addition,

cupric acetate-phosphoric acid was replaced by PMA as the detection reagent. Solvent 2 is the best mobile phase we have found to date for qualitative and quantitative determination of cholesterol esters in complex lipid mixtures. As can be seen in Figure 2, it also provides excellent resolution of triacylglycerols (R_f 0.32) and could be used for their densitometric quantification after dilution of the yolk extracts so that the scan areas of the samples were closely matched to the areas of the standards.

It was proven earlier (1) by use of preparative silica gel TLC to separate the sterol fraction followed by argentation- and RP-TLC and capillary gas chromatography to resolve and identify the individual sterols that cholesterol was the only sterol present in egg yolk extract. Therefore, cholesterol could be reliably quantified using a pure cholesterol standard. Similarly, pure standards of oleic acid, triolein, and cholesteryl oleate were used as markers and quantitative standards for analysis of free fatty acids, triacylglycerols, and cholesterol esters,respectively, in this study.

Calibration curves (peak area vs standard weight) prepared by spotting 2.0, 4.0, 8.0, 12.0, and 16.0 ul (400 ng to 3.20 ug) of standards for the four lipids quantified had typical linear correlation coefficients (R values) greater than 0.99. This strong linear correlation allowed quantification to be carried out reliably by comparing the areas of single sample and standard zones on the same plate if the areas of the two zones matched closely and were within the linear calibration range.

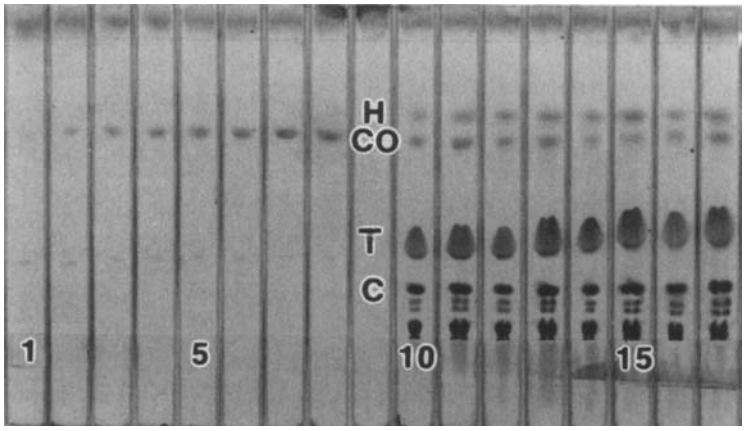

FIGURE 2. Photograph of chromatograms used for determination of cholesterol esters on a silica gel plate developed with solvent 2 and detected with PMA. Lanes 1-8 contain 1, 2, 3, 4, 5, 6, 7, and 8 ul aliquots of a 0.2 ug/ul standard solution of cholesteryl oleate (CO). Lanes 10 and 11, 12 and 13, 14 and 15, and 16 and 17 contain 9 and 15 ul, respectively, of yolk extract from four different regular eggs, in which zones of hydrocarbons (H), cholesterol esters (CO), triacylglycerols (T), cholesterol (C), and the more polar lipids and phospholipids below cholesterol can be seen.

Besides the four lipid fractions quantified, additional faint zones were found in yolk chromatograms developed with solvent 1 at R_f values of 0.32, 0.40, and 0.92. These zones probably represented monoacylglycerols, diacylglycerols, and nonpolar hydrocarbons, respectively. Phospholipid zones were also detected in the preadsorbent and streaking just beyond the preadsorbent-silica gel interface. With solvent 2, nonpolar hydrocarbons were detected above the cholesterol esters at R_f 0.76 (Figure 2). In our earlier analyses of egg eggs using the Skipski solvent system, free fatty acids were

not identified in yolk samples as they were in the present study. Methyl oleate, the marker for methyl esters, was resolved between the cholesterol oleate and triolein standards (Figure 1) but was not detected in egg yolk chromatograms in either this or the earlier study (1).

In summary, the HPTLC method described allows quantification of cholesterol, free fatty acids, triacylglycerols, and cholesterol esters in egg yolk with accuracy and precision that are adequate for routine analytical use. It is more selective than previously reported methods for lipid analysis based on spectrophotometry, and the ability to spot multiple samples on a single plate provides a rapid analysis with high sample throughput. Solvent consumption is very low because of the nature of the microextraction procedure and use of the twin trough chamber.

ACKNOWLEDGEMENT

MCS was supported by a Jean Boissevain Undergraduate Scholarship awarded to the Chemistry Department of Lafayette College by the Camille and Henry Dreyfus Foundation, Inc.

REFERENCES

1. K. Morris, J. Sherma, and B. Fried, J. Liq. Chromatogr. 10, 1277-1290 (1987)

2. C. Masterson, B. Fried, and J. Sherma, Microchem. J. 47, 134-139 (1993)

Received: July 26, 1994
Accepted: August 13, 1994