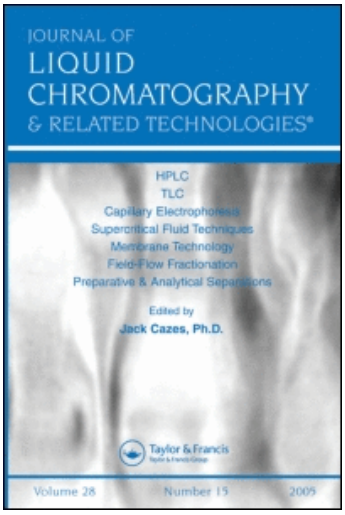

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effect of Hydrogen/Air Flow Rates and Scan Rate on the Flame Ionization Detection Response of Phospholipids, and their Qualitative and Quantitative Analysis by Iatroscan (Mark-6s) TLC-FID

W. M. Indrasena[a]; C. J. Barrow[a]; J. A. Kralovec[a]
[a] Ocean Nutrition Canada Ltd., Dartmouth, Nova Scotia, Canada

To cite this Article Indrasena, W. M. , Barrow, C. J. and Kralovec, J. A.(2006) 'Effect of Hydrogen/Air Flow Rates and Scan Rate on the Flame Ionization Detection Response of Phospholipids, and their Qualitative and Quantitative Analysis by Iatroscan (Mark-6s) TLC-FID', Journal of Liquid Chromatography & Related Technologies, 29: 14, 2111 — 2127
To link to this Article: DOI: 10.1080/10826070600759983
URL: http://dx.doi.org/10.1080/10826070600759983

Journal of Liquid Chromatography & Related Technologies®, 29: 2111–2127, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600759983

Effect of Hydrogen/Air Flow Rates and Scan Rate on the Flame Ionization Detection Response of Phospholipids, and their Qualitative and Quantitative Analysis by Iatroscan (Mark-6s) TLC-FID

W. M. Indrasena, C. J. Barrow, and J. A. Kralovec
Ocean Nutrition Canada Ltd., Dartmouth, Nova Scotia, Canada

Abstract: The combined effect of scan speed, hydrogen and air flow rates on the flame ionization detection (FID) peak response of phospholipid classes has been studied to determine the optimum levels of these parameters. The phospholipid composition of different types of commercial lecithins, as well as lecithins combined with fish oils, has been analyzed by Iatroscan TLC-FID Mark-6s under optimized conditions.

An air flow rate of 2 L/min, a hydrogen flow rate of 150–160 mL/min, and a scan speed of 30 s/rod seem to be the ideal conditions for scanning phospholipids with complete pyrolysis in the flame in the Mark-6 model. Increasing the scan speed rapidly decreased the FID response. A hydrogen flow rate as high as 170 mL/min could be used at relatively low air flow rates (<2 L/min) and the response declined when both air flow rate and hydrogen flow rate increased simultaneously. Both linear and curvilinear relationships had highly significant correlations ($p < 0.01$) with the sample load. Time course reactions, including the hydrolysis of phosphatidylserine using enzymes, can be successfully monitored by the Iatroscan TLC-FID Chromarod system.

Keywords: Lipid classes, Phospholipids, TLC-FID, Iatroscan-Mark-6s

Address correspondence to W. M. Indrasena, Ocean Nutrition Canada Ltd., 101, Research Drive, Dartmouth, Nova Scotia, Canada, B2Y 4T6. E-mail: windrasena@ocean-nutrition.com

INTRODUCTION

Iatroscan-Chromarod systems, combined with TLC-FID, have been commonly used for the determination of various organic substances, such as phospholipids, in a variety of samples including fats and oils.[1–3] FID response in this technology varies considerably with various environmental and physico-chemical factors, type and geometry of the detector in the system, as well as scan speed and hydrogen/air flow rates.

Recent technological advancement in the application of various kinds and forms of phospholipids in foodstuffs, as a result of their favourable biological and surface active properties, demands the development of methods for the analysis of these compounds which are widely distributed in nature. Classical TLC and HPTLC methods have been commonly used for the detection of phospholipids in various biological tissues and preparative TLC has been used for further analysis of individual phospholipid species.[4,5] Such methods have not been satisfactory for the quantification of phospholipid species in a mixture of multiple components of lipids. A complete analysis of individual neutral and polar lipid classes in any marine lipid sample can be achieved by the TLC-FID technology.[6–8]

Application of TLC-FID has been confined to the quantification of natural phospholipids, especially amphoteric phospholipids, due to the problems encountered in the amount of complex mixtures. Complex bioorganic phospholipid derivatives that have recently become quite important as neutraceuticals,[9,10] as well as non-toxic emulsifiers in a variety of applications in the food industry, have still not been analyzed by this technology. These complex mixtures contain salts of phosphatidic acids, glycerophospholipids, such as phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PEA), phosphatidylinositol (PI), and their positional lysoderivatives, in addition to neutral lipids, such as acyl glycerols, free fatty acids, and free sterols. Although the separation of such a large number of components is tedious and time consuming, sequential multiple development methods have been successfully used for the separation and detection of such components in biological tissues.[11–14]

The sensitivity of any compound to the FID is mainly based on the amount of ions generated over the flame during the pyrolysis of the band of lipid component separated on the rod. This ionization ability varies, depending upon the nature of the compound, as well as the hydrogen and air flow rates and the scan speed. The quantification of lipid classes can be complicated by a number of factors, including degree of volatility and variations in the amount of ionizable carbon produced during the pyrolysis of different lipid classes.[15]

The objective of the present work is mainly to calibrate the Iatroscan Mark-6s system for qualitative and quantitative analysis of polar lipids, such as phospholipids including phosphatidylcholine, phosphatidylethanolamine,

lysophosphatidylcholine, phosphatidylserine, lysophosphatidylserine, and phosphatidic acid.

EXPERIMENTAL

Effect of Scan Speed, Air and Hydrogen Flow Rates

All analyses were performed by TLC on Chromarods-SIII, using Iatroscan Mark-6s. Authentic standards of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, phosphatidic acid, lysophosphatidylcholine, lysophosphatidylethanolamine, and lysophosphatidylserine of >99% purity were purchased from Serdary Research Laboratories (Toronto). Commercial lecithin samples were generously provided by The Solae company (Solae, LLC., Indiana, USA.) and Danisco (Danisco Cultor, USA, New Century, KS.). Commercial phosphatidylserine was purchased from Enzymotec (Magdal Haemek, Israel).

Chromarods were activated by scanning them twice at a hydrogen flow rate of 170 mL/min, air flow rate of 2.0 L/min, and a scan speed of 30 s/rod prior to the application of samples. One microliter (1 μL) of each standard (3–10 mg/mL chloroform or chloroform/methanol), or a mixture of standard phospholipids was spotted onto the chromarods using Drummond Microcap disposable pipettes (Acadian Instrument Ltd., Etobicoke, ON, Canada), followed by solvent focusing with chloroform/methanol (1 : 1). Rods were then developed in chloroform:methanol:water:formic acid (65 : 35 : 2 : 0.04) for 50 min. The development tank was saturated with the solvent prior to the development.

Effect of Air and Hydrogen Flow Rates

Chromarods were scanned at a constant scan speed of 30 s/rod while hydrogen and air-flow rates were changed to see the effect of hydrogen and air flow-rates on the FID response of individual phospholipids. Hydrogen flow rate was gradually increased from 130 to 170 mL/min and air flow rate was increased from 1.0 to 3.0 L/min.

Effect of Scan Speed

The scan speed was changed while hydrogen and air flow rates were kept constant at the optimized levels obtained by scanning the phospholipid standards at different hydrogen and air flow rates. The scan speed was gradually increased from 25 to 60 s/rod at the constant hydrogen flow rate of 160 mL/min and air flow rate of 2.0 L/min to determine the effect of scan speed on the FID response of phospholipids.

Calibration of Chromarods

Chromarods were pre-scanned at a hydrogen flow rate of 170 mL/min, air flow rate of 2.0 L/min, and scan speed of 30 s/rod. One microliter of the mixture of standard phospholipids with varied concentrations (0.5–6 mg/mL) were spotted onto the rods and developed in hexane:diethyl ether:formic acid (HDF)(90 : 10 : 0.04) for 50 min. After the partial scanning at a hydrogen flow rate of 160 mL/min, air-flow rate of 2.0 L/min, and a scan speed of 30 s/rod, the rods were developed in chloroform:methanol:water:formic acid (65 : 35 : 2 : 0.04) for 50 min. They were subsequently scanned completely at the hydrogen flow rate of 160 mL/min, air flow rate of 2.0 L/min, and scan speed 30 s/rod.

Qualitative and Quantitative Analysis of Phospholipids

Samples consisting of fish oil and phospholipids, phosphatidylserines, and commercial lecithins were analyzed by a multiple developmental procedure.

Chromarods were pre-scanned at a hydrogen flow rate of 170 mL/min, air flow rate of 2.0 L/min, and scan speed of 30 s/rod. Individual samples with a concentration of 5–10 mg/mL were spotted, focused with chloroform:methanol (3 : 1) and developed in hexane:diethyl ether:formic acid (90 : 10 : 0.04) for 50 min. After drying in the air for about 10 min, they were partially scanned (about 70% of the total length of the rod) to the base behind the last neutral lipid peak at a hydrogen flow rate of 160 mL/min, air-flow rate of 2.0 L/min, and a scan speed of 30 s/rod. Rods were then developed in 100% acetone for 20 min, dried in the air for about 10 min, and partially scanned (about 70% of the total length of the rod). Final development was performed in chloroform:methanol:water:formic acid (65 : 35 : 2 : 0.04) for 50 min, dried at 110°C for 3–5 min and completely scanned (the full length of the rods) under the same conditions.

Thin-Layer Chromatography

Phospholipid samples were screened by TLC prior to the application to TLC-FID by the Iatroscan Chromarod system. Samples, as well as corresponding commercial phospholipid standards, with a concentration of 5–10 mg/mL, were spotted onto aluminum-backed Silica TLC sheets (Silica gel 60 F254), developed in chloroform:methanol:water:formic acid (65 : 35 : 2 : 0.04) for 10 min, and dried at room temperature for about 10 min. The phospholipid spots were then detected using 2′-7′-dichlorofluoresceine under UV light. Phosphatidylserine and lysophosphatidylserine were detected using alcoholic ninhydrin reagent.

RESULTS

Hydrogen and Air Flow Rates

At a constant scan speed of 30 s/rod, the FID responses of all phospholipids increased with increasing air flow rate. The FID response increased with increasing hydrogen flow rate up to 150 mL/min and then declined gradually, irrespective of the air flow rate (Figs. 1–5).

Scan Speed

At constant hydrogen and air flow rates, the FID responses of all compounds initially increased up to the scan speed of 30 sec/rod and then rapidly decreased with further increases of scan speed (Fig. 6).

Calibration Curves

FID response for all phospholipids showed linear relationships with increasing sample load, except for phosphatidic acid (PA) (Figs. 7A–E).

Qualitative and Quantitative Analysis

Most of the phospholipids were separated using the chloroform:methanol:water:ormic acid based solvent systems. Figures 8–11 show the TLC-FID chromatograms of some commercial lecithins, as well as mixtures of lecithin and fish oil. The production of LPS from PS during the hydrolysis with phospholipase-A2 is shown in Fig. 12.

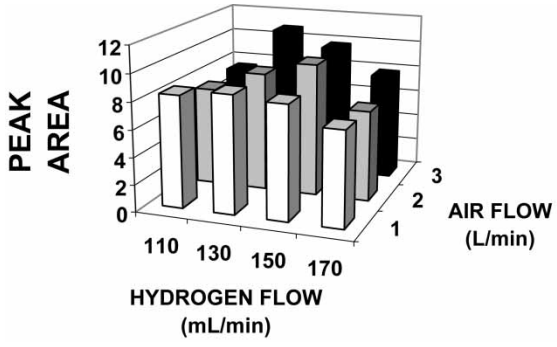

Figure 1. Effect of hydrogen and air flow rates on the FID response of phosphatidylserine.

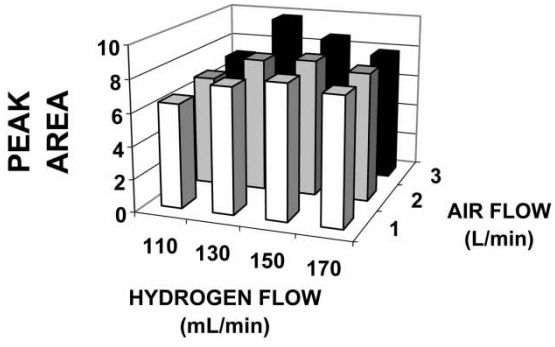

Figure 2. Effect of hydrogen and air flow rates on the FID response of phosphatidic acid.

DISCUSSION

The FID response is based on the amount of ions generated in the flame in the presence of air and hydrogen during the pyrolysis of lipid bands separated on the silica rod. This actually measures the change in the ionization current resulting from a sudden increase in the ion density flowing between the burner (the 'cathode') and the collector electrode (the 'anode') after the introduction of an analyzed component into the hydrogen-oxygen flame.[4] The generation of ions entirely depends upon the basic structure of the compound. Chemionization and thermionization are the primary ionization processes that affect the ionization current. Chemionization happens in a very narrow region of oxygen rich area of the flame, at a point where intimate mixing of hydrogen and oxygen occurs. Free electrons, OH- anions, and H_3O^+ ions are formed from the reaction of neutral H, O and OH species in the flame, whereas CHO^+, CH_3O^+, $C_3H_3^+$, C_2^-, O^-, C_3^-,

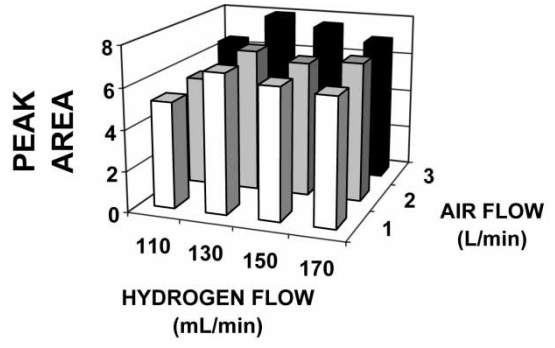

Figure 3. Effect of hydrogen and air flow rates on the FID response of lysophosphatidylserine.

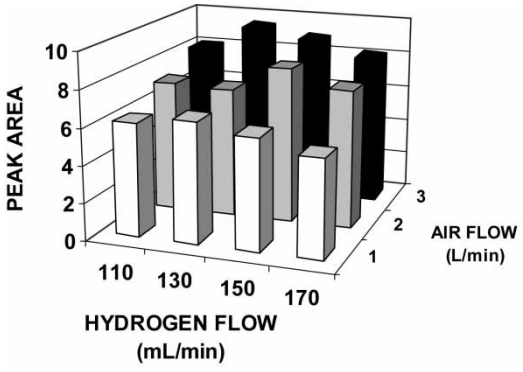

Figure 4. Effect of hydrogen and air flow rates on the FID response phosphatidylcholine.

CO_2^- are formed by the burning of hydrocarbons.[4] Burning of phospholipid species containing long-chain fatty acids ($C_{16}-C_{22}$), choline, serine, as well as free amino groups, should produce many other ions as well those that affect the ionization current and, subsequently, the FID response. However, irrespective of the basic structures of different phospholipid molecules, all phospholipids demonstrated very similar trends of FID responses for hydrogen and air-flow rates at a given scan speed.

The formation of detectable ions usually increases with increasing air and hydrogen flow rates to a certain degree, depending upon the type of material, as well as sample load.[4,16] However, the upper limits of hydrogen flow rate depend upon the scanning rate, as well as on the air flow rate. At a moderate scan speed of 30 s per rod, the FID responses of all phospholipids steadily

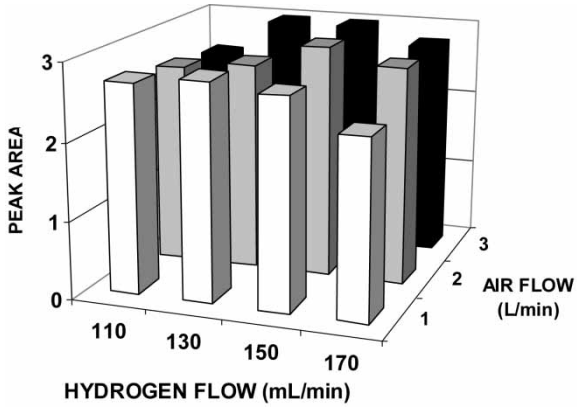

Figure 5. Effect of hydrogen and air flow rates on the FID response of lysophosphatidylcholine.

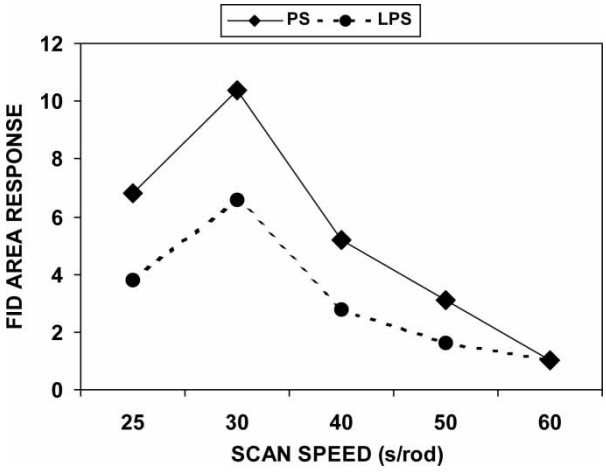

Figure 6. Effect of scan speed on the FID response (PS = phosphatidylserine, LPS = lysophosphatidylserine).

increased with increasing air flow rate, with a simultaneous increase of hydrogen flow rate up to 130–150 mL/min. Further increase of hydrogen flow rate with simultaneous increase of air flow rate did not improve the FID response. This may be due to rapid vapourization and disappearance of ions from the flame, especially at low loading levels. At the air flow rate of 3 L/min, the highest FID response could be obtained even with the hydrogen flow rate of 130 mL/min; this is more pronounced with phosphatidylserine, lysophosphatidylserine, and phosphatidic acid (Figs. 1–3). In order to improve the FID response at the air flow rate of 2 L/min, the hydrogen flow rate could be increased up to 150–160 mL/min, simultaneously. At higher loading levels, higher hydrogen flow rates may be required under otherwise similar conditions.

The temperature of the reaction zone of the flame rapidly increases with a simultaneous increase of hydrogen and air flow rates, resulting in rapid ionization, as well as rapid vapourization of the molecules. Therefore, if the sample load is very low, maximum ionization could happen at the air flow rate of 2 L/min with a simultaneous hydrogen flow rate of 130 mL/min. However, it is safer to use hydrogen flow rates of 160 mL/min to assure the complete combustion of phospholipids in the flame. It was clear that individual phospholipids had different FID responses, although the conjugated fatty acids in the phospholipid molecules and concentrations were the same. In previous Iatroscan models, in which the detector geometry was different from the latest version of Iatroscan Mark-6s, the highest FID responses for some neutral lipids in the range of 0.38–0.43 μg were obtained at hydrogen flow rates of 150–200 mL/min and maximum hydrogen flow rate of 180 mL/min even has been recommended for those models.[17] In previous Iatroscan models,

the detector consisted of a stationary collector electrode (anode) and a burner (cathode) in which the distance could be adjusted by a screw. This distance seems too large and can cause detrimental effect on the uniformity of the electric field.[4] The improvement of the geometry in the latest Iatroscan model, Mark-6s, has a considerable positive effect on the ionization current, since the maximum FID response can be obtained even at a hydrogen flow

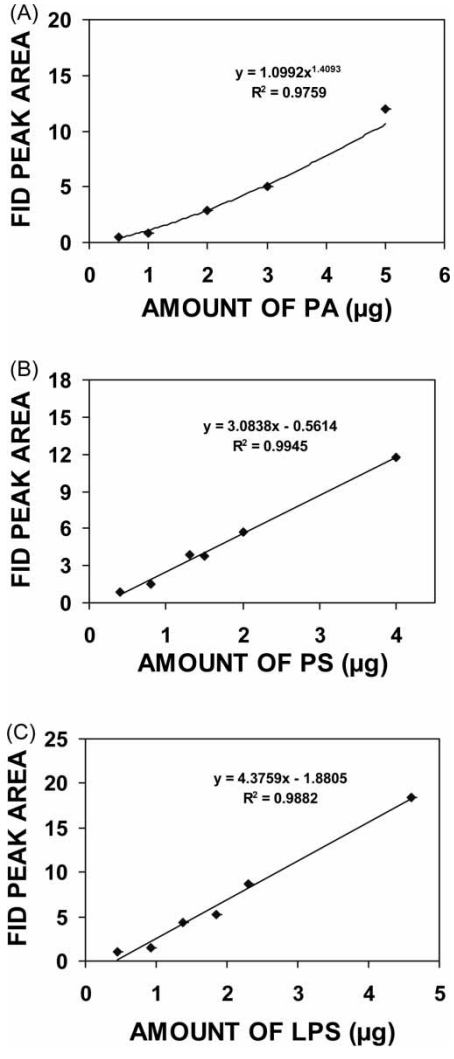

Figure 7. A. Calibration curve for phosphatidic acid; B. Calibration curve for phosphatidylserine; C. Calibration curve for lysophosphatidylserine; D. Calibration curve for phosphatidylcholine; E. Calibration curve for lysophosphatidylcholine.

(*continued*)

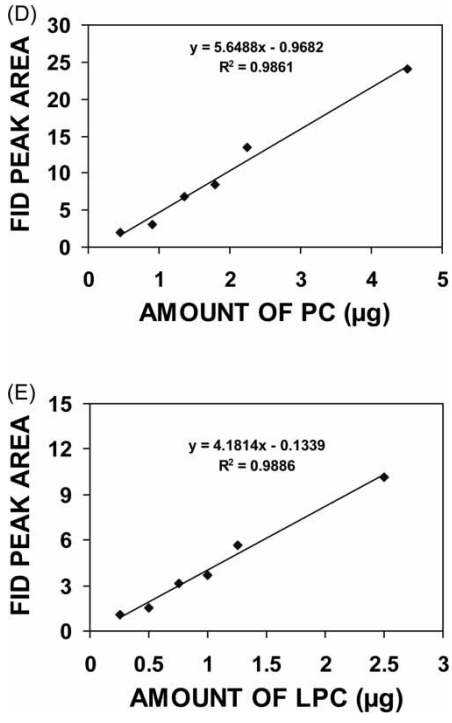

Figure 7. Continued.

rate as low as 130–150 mL/min, especially at low loading levels. A high FID response for neutral lipids, also using relatively low hydrogen flow rates (130–150 mL/min) in this model, with the simultaneous air flow rate of 2 L/min, and scan rate of 30 s/rod, has been reported previously.[16] A decrease in the air flow rate with a simultaneous increase in the hydrogen flow rate has a considerable effect on the FID responses of substances with lower carbon contents and higher halogen contents, since the detector response of hydrogen-halides increases while decreasing the carbon, with increasing hydrogen flow rate.[18,19] The phosphorus in phospholipids may play a different role in the ionization current besides the basic structure of different phospholipids species. The amount of air passing through the detector of previous models could not be measured precisely, whereas, it can be well adjusted to the required amount, up to 3.0 L/min, in both the Mark-5[20] and the Mark-6s models, in which the variability in the FID response resulting by even minute fluctuations of air flow while scanning could be minimized. The possibility of using lower hydrogen flow rates is another advantage of the Mark-6 model.

The Iatroscan Mark-6s TLC-FID system has a feature of numerically changing the velocity of the rods from 25 to 60 s/rod. The FID responses of

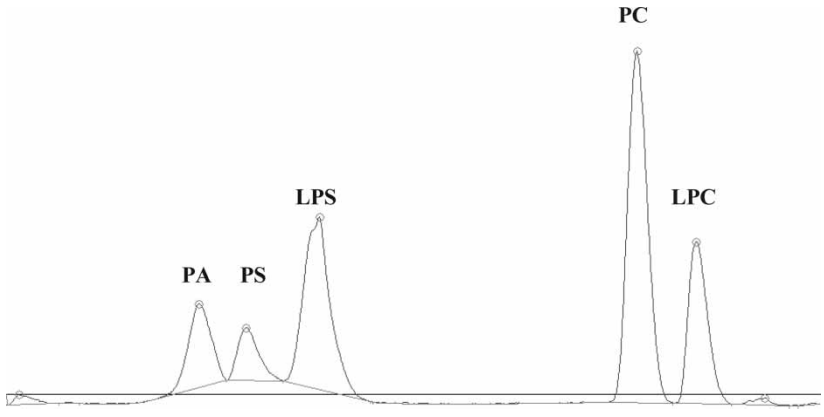

Figure 8. TLC-FID chromatogram of a mixture of phospholipids (PA = phosphatidic acid, PS = phosphatidylserine, LPS = lysophosphatidylserine, PC = phosphatidylcholine, LPC = lysophosphatidylcholine).

all phospholipids dramatically decreased with increasing scan speed at constant hydrogen and air- flow rates. Slow movement of the rods in the flame leads to heating of a broader zone of the rod, thus resulting in the volatilization of a greater fraction of the analyzed mixture. The ionization current is proportional to the scan speed at which the substance enters the flame and FID response is proportional to the ions generated in the flame. The degree of evaporation depends mainly on the flame temperature and rod conductivity, as well as the volatility of the compound. However, excessively high scanning rates seem to have serious adverse effects, not only on the ionization current and, subsequently, the FID response, but also on the effective lifetimes of the silica rods.[4,16]

Separation of all phospholipids in a natural oil sample using a single solvent system is not feasible, especially when other non-polar lipids are

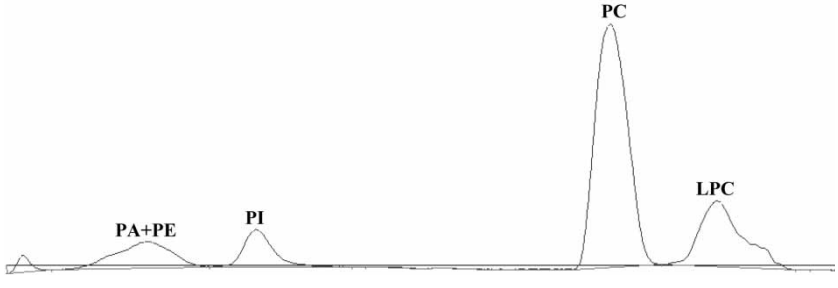

Figure 9. TLC-FID chromatogram of a commercial lecithin type-1 (PA = phosphatidic acid, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PC = phosphatidylcholine, LPC = lysophosphatidylcholine).

Figure 10. TLC-FID chromatogram of a commercial lecithin type II (PA = phosphatidic acid, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PC = phosphatidylcholine, LPC = lysophosphatidylcholine).

present in the sample. Therefore, sequential development, which is unique to the Iatroscan TLC-FID system, must be used. After resolving non-polar lipids with HDF systems, phospholipids could be separated with a chloroform: methanol:water:formic acid (CMWF) based solvent system. All standards and the mixtures of standards could be directly developed in a chloroform: methanol:water system, since they do not contain any neutral lipid groups. Since phosphatidylserine which has been trans-esterified with fish oil contained a considerable amount of free fatty acids or ethyl esters from the

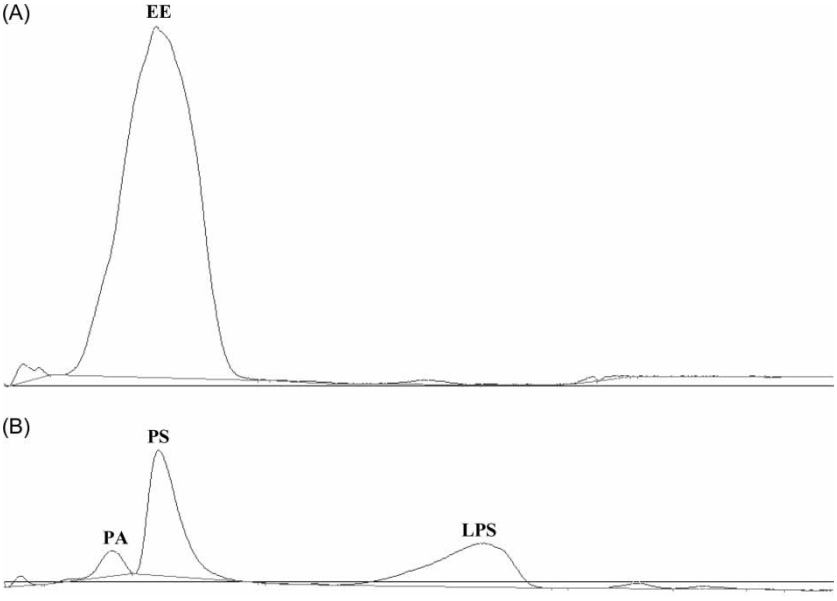

Figure 11. a) TLC-FID chromatogram of fish oil containing PS after first development in HDF system (EE = ethyl esters); b) TLC-FID chromatogram of fish oil containing PS after final development in CMWF system (PA = phosphatidic acid, PS = phosphatidylserine, LPS = lysophosphatidylserine).

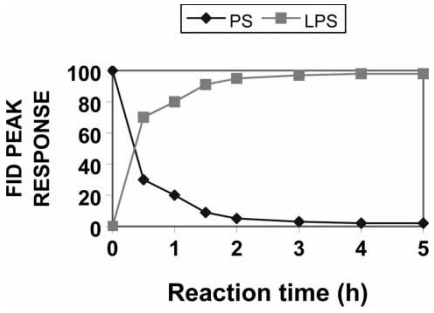

Figure 12. Hydrolysis of phosphatidylserine at 50°C (PS = phosphatidylserine, LPS = lysophosphatidylserine).

fish oil, the rods were initially developed in an HDF system to elute all neutral lipids, then in acetone to elute any acetone-mobile polar lipids which are less polar than phospholipids and, finally, in a chloroform:methanol:water system to elute phospholipids. However, samples of phospholipids separated from silica-gel cartridges were developed directly in the chloroform:methanol: water system, since all neutral lipids were initially separated from the column prior to the extraction of phospholipids. PS, PA, and LPS eluted quite closely and their peaks were not very sharp, but short and broad with tailing. This may be probably due to the variability of the compositions of fatty acids attached to the PS backbone. Broad and tailing peaks in some triacylglycerols and free fatty acids in marine oils could be attributed to the nature of unsaturation of the compound, especially when larger percentages of polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are attached to the glycerol backbone,[21] and could be substantially reduced by exposing the developed chromarods to iodine vapour.[22]

A blend of commercial standards of LPS, PA, PS, PC, and LPC could be well separated in the chloroform:methanol:water system. PE and PI could not be used in the same mixture, due to the fact that, either they eluted very closely in the region of PA, PS, and LPS, and/or some over-lapping peaks. Therefore, in order to separate a natural mixture of a sample containing all these phospholipids, either the solvent system may be modified or the active surface of the chromarods may be modified to obtain narrower, sharper peaks that subsequently should improve the resolution. Although oxalic acid impregnated chromarods have been used for better resolution and peak shapes,[11,23] it is not a convenient method for quick, routine analysis of a large number of samples, especially in industry-oriented research, since the impregnation-cleaning is a time consuming process. Use of phosphorus-specific detectors such as the flame photometric detector could be the best option for the improvement of sensitivity.[5] Although LPS, PA, PS, PC, LPC, and LPEA were well separated with this solvent system at low loading levels, the resolution of PA and PS was poor at high loading levels, since they eluted closely.

Relatively high loads of PC, LPC, and LPEA can be easily separated in this mixture. PE and PI could not be used in the same mixture, since they co-elute or partially overlap. Also, all of these phospholipids in a single mixture could not be well separated by the chloroform:methanol:water system.

Peak area responses of the FID against the sample load of all phospholipids were highly significant ($p < 0.01$), with slightly higher coefficients of determination (R^2) for linear models than for curvilinear models. Therefore, linear models could be used for quantification of phospholipids, thereby avoiding complicated derivation of calibration equations. Linear calibration equations could be successfully used in the quantification of crude oil and hydrocarbons by the Mark-5,[24] as well as lipid classes in marine oils using the Iatroscan model Mark-6s.[16] Both conventional Power Law models ($y = ax^b$) and linear models have been used in the quantification of general lipid classes and the linearity of the FID response seems to have improved with the improvement of the Iatroscan model. However, although Power Law models have been used in most analyses by Iatroscan Mark-III and IV models,[25] in the separation and quantification of phospholipids in animal tissues using oxalic acid impregnated chromarods, FID of the Mark-II demonstrated a highly significant linear response with sample loads of all phospholipids, including LPC, PA, PS, PI, PE, and PC.[11] Therefore, the linearity may not totally depend on the geometry of the location of the detector, but on many other factors, such as chamber saturation, humidity of the environment, as well as the physico-chemical characteristics of the active surface of the chromarods. The FID response can be considerably affected by the developing solvents as well[26] and, in the present study, chamber saturation and solvent focusing showed a great influence on the peak shape and resolution of phospholipids. When linear models are used, it is important to note that these models should be applied only within the range in which the corresponding standards lie.[27] Otherwise, suitable calibration equations explaining the entire region of sample load must be used. Sample components with peak areas corresponding to sample loads below 1 mg/g are preferable to quantify using other models such as Power Law models, since the FID responses below this concentration levels are not explained by linear models. Extensive calibration equations that include the addition of quadratic or cubic terms or both may be appropriate in the quantification of a vast range of sample loads[28] using a single calibration equation. Since all samples were within the linear range of the calibration equations, linear models were used in quantifying the phospholipids in the present study. Humidity in the lab environment and saturation of the developing chamber showed a considerable effect on the resolution and peak shapes of phospholipids.

Both commercial lecithins contained about 35–40% PC. PA, LPC, PE, and PI were also present in these lecithin samples. TLC-FID was used for monitoring the reaction kinetics of the production of lysophosphatidylserine

during the hydrolysis of phosphaditylserine in the presence of phospholipases, as well as during the transesterification of phosphatidylserine with fish oils containing about 55% DHA. Figure 12 shows the production of lysophosphatidylserine during the hydrolysis of phosphatidylserine in the presence of phospholipase-A_2. The FID response varied considerably among different phospholipids and individual calibration equations were used to quantify the phospholipids. Lysophosphatidylserine content increased in a dramatic exponential manner, initially, and then reached a plateau after almost 2 h, whereas phosphatidylserine declined markedly to form lysoderivatives. Since phospholipase-A_2 is specific for the sn-2 position of the phospholipid molecule, the main product after hydrolysis is lysophosphatidylserine with a hydroxyl group at the sn-2 position. It is quite evident that the TLC-FID technique could be successfully used for monitoring both hydrolysis and transestierification of phospholipids. However, area percentage alone may not be a good choice for monitoring reaction kinetics, due to the variation in FID response, and the simplest, appropriate form of calibration equations should be used for quantification.

CONCLUSIONS

Flow rates of hydrogen and air, as well as the scan speed, considerably affect the FID response of lipid components separated on Chromarods. A hydrogen flow rate of 150–160 mL/min, an air flow rate of 2 L/min, and a scan speed of 30 s/rod were found to be the optimized operational conditions for the scanning of phospholipids by the Mark-6s model. Although basic and common phospholipids can be well separated by the CMW system, an addition of minute amounts of formic acid seem to help the resolution of phospholipid species. Linear calibration equations can be used for the quantification of phospholipids if the sample loads are within the corresponding load ranges of the standards.

REFERENCES

1. Ackman, R.G.; McLeod, C.A.; Banerjee, A.K. An overview of analyses by chromarod-Iatroscan TLC-FID. J. Planar Chromatogr. **1990**, *3*, 450–490.
2. Stirby, L.; Lafont, R.; Goutx, M. Improvements in the Iatroscan thin-layer chromatographic-flame ionization detection analysis of marine lipids Separation and quantitation of monoglycerols and diacylglycerols in standards and natural samples. J. Chromatogr. A **1999**, *849*, 371–380.
3. Hazel, J.R. Determination of the phospholipid composition of trout gill by Iatroscan TLC/FID: Effect of thermal acclimation. Lipids **1985**, *20*, 516–520.
4. Ranny, M. Methods of thin-layer chromatography with flame ionization detection. In *Thin-Layer Chromatography with Flame Ionization Detection*; Janåk, J., Ed.; D. Reidel Publishing Company, 1987; 30–83.

5. Ogasawara, M.; Tsuruta, K.; Arao, S. Flame photometric detector for thin-layer chromatography. J. Chromatogr. A **2002**, *973*, 151–158.
6. Kaitaranta, J.K.; Ackman, R.G. Total lipids and lipid classes of fish roe. Compt. Biochem. Physiol. **1981**, *69B*, 725–729.
7. Fraser, A.J.; Tocher, D.R.; Sargent, J.R. Thin-layer chromatography-flame ionization detection and the quantitation of marine neutral lipids and phospholipids. J. Exp. Mar. Biol. Ecol. **1985**, *88*, 91–99.
8. Aguilera, Y.; Dorado, M.E.; Prada, F.A.; Martinez, J.J.; Quesada, A.; Ruis-Gutierrez, V. The protective role of squalene in alcohol damage in chick embryo retina. Exptl. Eye Res. **2005**, *80*, 535–543.
9. Jorrissen, B.I.; Brouns, F.; VanBoxtei, M.F.J.; Reidel, W.J. Safety of soy derived phosphatidylserine in elderly people. Nutr. Neurosci. **2002**, *5*, 337–343.
10. Mozi, R.; Buratta, S.; Goracci, G. Metabolism and functions of phosphatidylserine in mammalian brain. Neurochem. Res. **2003**, *28*, 195–214.
11. Schrijiver, R.D.; Vermeulen, D. Separation and quantification of phospholipids in animal tissues. Lipids **1991**, *26*, 74–76.
12. Indrasena, W.M.; Parrish, C.C.; Paulson, A.T.; Ackman, R.G. A comparison of Alumina and Silica gel Chromarods for the separation and characterization of lipid classes by Iatroscan TLC-FID. J. Planar. Chromatogr. **1990**, *4*, 182–88.
13. Kaitaranta, J.K.; Nicolaides, N. Response and linearity of different lipid compounds when analyzed by thin-layer chromatography with flame ionization detection. J. Chromatogr. **1981**, *205*, 339–347.
14. Parrish, C.C.; Ackman, R.G. Calibration of the Iatroscan-Chromarod System for Marine Lipid Class Analyses. Lipids **1985**, *20*, 521–530.
15. Sipos, J.C.; Ackman, R.G. Automated and rapid quantitative analysis of lipids in ripe roes of some northwest European marine fish. J. Chromatogr. Sci. **1978**, *16*, 443–447.
16. Indrasena, W.M.; Henneberry, K.; Barrow, C.J.; Kralovec, J.A. Qualitative and quantitative analysis of lipid classes in fish oils by Thin-Layer-Chromatography with an Iatroscan Flame Ionization Detector (TLC-FID) and liquid chromatography with evaporative Light Scattering Detector (LC-ELSD). J. Liq. Chromatogr. Rel. Technol. **2005**, *28*, 2581–2595.
17. Bradley, D.M.; Rickards, C.R.; Thomas, N.S.T. Plasma lipid analysis by thin-layer chromatography with flame ionization detection and quantitation. Clin. Chim. Acta **1979**, *92*, 293–302.
18. Russev, P.; Gough, T.A.; Wollam, C.J. Detection of inorganic gases using a flame ionization detector. J. Chromatogr. **1976**, *119*, 461–466.
19. Karagözler, A.E.; Simpson, C.F. Direct quantitative analysis using the flame-ionization detector. J. Chromatogr. **1978**, *150*, 329–338.
20. Indrasena, W.M.; Ackman, R.G.; Gill, T.A. Separation of paralytic shellfish poisoning toxin on Chromarods-S III by thin layer chromatography with Iatroscan Flame Thermionic Detection (FTID). J. Chromatogr. A **1999**, *855*, 657–668.
21. Oshima, T.; Ratnayake, W.M.N.; Ackman, R.G. Cod lipids, solvent systems and the effect of fatty acid chain length and unsaturation on lipid class analysis by Iatroscan TLC-FID. J. Am. Oil Chem. Soc. **1987**, *64*, 219–223.
22. Banerjee, A.K.; Ratnayake, W.M.N.; Ackman, R.G. Enhance response of more highly unsaturated lipids on thin-layer chromatography-Iatroscan Flame Ionization Detection after exposing the developed Chromarods to iodine vapour. J. Chromatogr. **1985**, *319*, 215–217.

23. Banerjee, A.K.; Ratnayake, W.M.N.; Ackman, R.G. Effect of oxalic acid impregnation of Chromarods on the separation of phospholipids for determination by the Iatroscan TLC/FID. Lipids **1965**, *20*, 121–125.
24. Bharathi, S.; Røstum, G.A.; Løberg, R. Calibration and standardization of Iatroscan (TLC-FID) using standards derived from crude oils. Adv. Org. Chem. **1993**, *22*, 835–862.
25. Parrish, C.C. Separation of aquatic lipid classes by Chromarod Thin-Layer Chromatography with measurements by Iatroscan Flame Ionization Detection. Can. J. Fish. Aquat. Sci. **1987**, *44*, 722–731.
26. Parrish, C.C.; Ackman, R.G. The effect of developing solvents on lipid class quantification in Chromarod Thin Layer Chromatography/Flame Ionization Detection. Lipids **1963**, *18*, 563–565.
27. Sebedio, J.; Juaneda, P. Quantitative lipid analyses using the new Iatroscan TLC-FID system. J. Plan. Chromatogr. **1991**, *4*, 35–41.
28. Indrasena, W.M. Separation of lipid classes by Thin Layer Chromatography on Chromarods- A and Chromarods-SIII with Iatroscan Flame Ionization Detection. M. Sc. Thesis, Dept. of Food Science and Technology, Technical University of Nova Scotia (Dal Tech), Halifax, Nova Scotia, Canada, 1992.

Received December 23, 2005
Accepted January 13, 2006
Manuscript 6864K