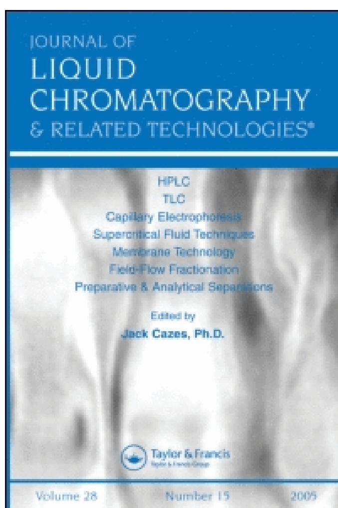
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

ONE-STEP SEPARATION SYSTEM FOR THE MAIN PHOSPHOLIPIDS, GLYCOLIPIDS, AND PHENOLICS BY NORMAL PHASE HPLC. APPLICATION TO POLAR LIPID EXTRACTS FROM OLIVE AND SUNFLOWER OILS

T. Nomikos[a]; H. C. Karantonis[a]; E. Fragopoulou[a]; C. A. Demopoulos
[a] Faculty of Chemistry Panepistimioupolis, National and Kapodistrian University of Athens, Athens, Greece

Online publication date: 01 November 2002

To cite this Article Nomikos, T. , Karantonis, H. C. , Fragopoulou, E. and Demopoulos, C. A.(2002) 'ONE-STEP SEPARATION SYSTEM FOR THE MAIN PHOSPHOLIPIDS, GLYCOLIPIDS, AND PHENOLICS BY NORMAL PHASE HPLC. APPLICATION TO POLAR LIPID EXTRACTS FROM OLIVE AND SUNFLOWER OILS', Journal of Liquid Chromatography & Related Technologies, 25: 1, 137 — 149

To link to this Article: DOI: 10.1081/JLC-100108545
URL: http://dx.doi.org/10.1081/JLC-100108545

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 25(1), 137–149 (2002)

ONE-STEP SEPARATION SYSTEM FOR THE MAIN PHOSPHOLIPIDS, GLYCOLIPIDS, AND PHENOLICS BY NORMAL PHASE HPLC. APPLICATION TO POLAR LIPID EXTRACTS FROM OLIVE AND SUNFLOWER OILS

Downloaded At: 09:13 24 January 2011

T. Nomikos, H. C. Karantonis, E. Fragopoulou, and C. A. Demopoulos*

National and Kapodistrian University of Athens, Faculty of Chemistry, Panepistimioupolis, 15771 Athens, Greece

ABSTRACT

A new normal phase high performance liquid chromatographic system has been developed that allows efficient separation of several polar lipids, such as phospholipids, glycolipids, and phenolics.

By this method, hesperidin, naringin, cerebrosides, phosphatidylcholine, sphingomyelin, platelet activating factor, lysophosphatidylcholine, digalactosyldiglycerides, and phosphatidylethanolamine are separated within 60 min.

A gradient elution with acetonitrile/methanol, methanol, and water was performed with a normal phase aminopropyl-

*Corresponding author. 39 Anafis Str., Athens, GR 113 64, Greece; E-mail: demopoulos@chem.uoa.gr

Copyright © 2002 by Marcel Dekker, Inc. www.dekker.com

ORDER REPRINTS

modified silica gel HPLC column. The eluted lipids were monitored by UV detection.

In addition, in this study, a successful application of the new method is represented for the polar lipids of olive oil and sunflower, which are also identified with chemical determinations.

INTRODUCTION

Polar lipid extracts from natural sources contain a wide variety of complex compounds of different polarities, such as phospholipids, glycolipids, and pigments including phenolic derivatives. Several chromatographic steps are required to separate such mixtures due to its complex molecular heterogeneity. (1–9) Traditionally, the separation and analysis of polar lipid extracts were carried out by column chromatography and one or two-dimensional thin-layer chromatography. (10) However, in the last two decades high-performance liquid chromatography has been considered to be the most efficient chromatographic technique for lipid separations due to its simplicity, high resolution power, and recovery yields, and wide range of column packing materials. (11–18) Although silica gel is the most common packing material for normal phase HPLC polar lipid analysis, (19–21) to our knowledge only few researchers have used aminopropyl-modified silica gels for lipid separation. (22,23)

The analysis of lipids from edible vegetable oils is of great importance in order to estimate their nutritional value. Although most of the studies are focused on the composition of the neutral lipids and the fatty acid profile of them, (24–26) the polar lipids are far less studied despite the fact that some of their minor constituents seem to have significant biological activities. (4)

This study demonstrates the utilization of an aminopropyl-modified silica gel HPLC column for the one step separation of the main classes of phospholipid, glycolipids, and phenolics, and its application for the analysis of polar lipid fractions from edible vegetable oils.

EXPERIMENTAL

Materials and Reagents

All reagents were of analytical grade and supplied by Merck (Darmstadt, Germany). HPLC grade solvents were purchased from Ruthburn (Walkerburn, Peebleshire, UK). Phospholipid, glycolipid, and phenolic standards were obtained from Sigma (St. Louis, MO, USA). [^{3}H] PAF (NET 910) was Purchased from

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

NEN (Dupont, Boston, MA). Chromatographic material used for analytical thin-layer chromatography was silica gel 60-H (Merck, Darmstadt, German). Vegetable oils were purchased from the local market.

Preparation of Standard and Samples

Phenolics were prepared as follows: Naringin was prepared as ethanolic solution with a concentration of 1 μg/μL. Hesperidin was prepared as methanolic solution with a concentration of 0.5 μg/μL. All phospholipid and glycolipid standards were prepared as 1% solutions in chloroform/methanol (1:1).

Polar lipid fractions were isolated from the two vegetable oils (olive oil and sunflower oil) by a modified method of Galanos and Kapoulas, (27) which will be described elsewhere [unpublished data]. Briefly, an amount of vegetable oil is diluted in a quadruple volume of petroleum ether (b.p. 40–70°C) and the whole mixture is washed with 87% ethanol several times. Afterwards, the combined ethanol phases are washed with petroleum ether several times. Finally, the combined petroleum phases are washed with ethanol. The combined ethanol phases contain polar lipids in a yield of 96%, while the combined petroleum phases contain neutral lipids.

Downloaded At: 09:13 24 January 2011

Instrumentation

Radioactivity was measured in a 1209 RackBeta-Flexivial β-Counter (LKB-Pharmacia, Turku, Finland).

Separation was performed on a HP HPLC Series 1100 liquid chromatography model (Hewlett Packard, Waldbronn, Germany) equipped with a 100 μL loop Rheodyne (7725 i) loop valve injector, a degaser G1322A, a quat gradient pump G1311A, and a HP UV spectrophotometer G1314A as a detection system. The spectrophotometer was connected to a Hewlett-Packard (Hewlett Packard, Waldbronn, Germany) model HP-3395 integrator-plotter.

A normal phase column, Sphereclone 5 μNH_2, 25 cm × 4.6 mm (I.D.), Phenomenex, (Hurdsfield Ind. Est., UK) was used.

HPLC and TLC Analysis

Volumes 5–20 μL of standard solutions containing 20–100 μg of polar lipids were injected each time. The flow rate was 1 mL/min and the eluted substances detected spectrophotometrically, using UV detection at 208 nm for phospholipids and glycolipids and 280 nm for phenolics. [^{3}H] PAF elution was detected by measuring the radioactivity of one-minute fractions collected from

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Table 1. Retention Times (RTs) of Individual Standards

Peak Number	Standard Lipids	Molecular Class	RT (min)
1	Hesperidin	Phenolic	7.73
2	Cerebrosides	Glycolipid	8.63
3	Naringin	Phenolic	9.88
4	Cerebrosides	Glycolipid	11.83
5	Phosphatidylcholine	Phospholipid	17.63
6	Sphingomyelin	Phospholipid	23.88
7	PAF[a]	Phospholipid	25.00
8	Lysophosphatidylcholine	Phospholipid	35.99
9	Digalactosyldiglyceride	Glycolipid	41.54
10	Sulfatides	Glycolipid	42.92
11	Phosphatidylethanolamine	Phospholipid	45.84

[a]Platelet activating factor.

Downloaded At: 09:13 24 January 2011

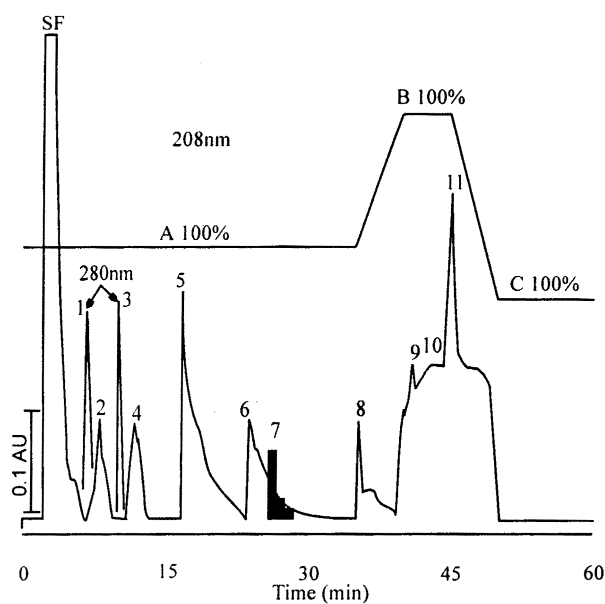


Figure 1. HPLC chromatogram(s) of polar lipid standards. Conditions and solvents A, B, and C in experimental section. Gradient as indicated. Peak identification in Table 1. SF: solvent front.

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

the column outlet. All chromatographic separations were carried out at room temperature.

TLC of column eluates was performed on 20 × 20 cm glass plates precoated with Silica gel H 0.5 mm thick. The plates were developed with chloroform/acetone/methanol/glacial acetic acid/water (50 : 20 : 10 : 10 : 5) (28). After the development, the solvent was evaporated and the plates were sprayed with a-naphthol stain (29) for the detection of glycolipids (blue purple spots) and phospholipids (yellow spots). Finally, for a more sensitive detection of lipids, the TLC plates were charred after spraying with concentrated sulfuric acid (black spots).

Chemical Determinations

Lipidic phosphorus was determined according to the method of Barlett. (30) Ester determination was performed according to the method of Renkonen. (31) Hexose determination was carried out according to Galanos and Kapoulas. (32)

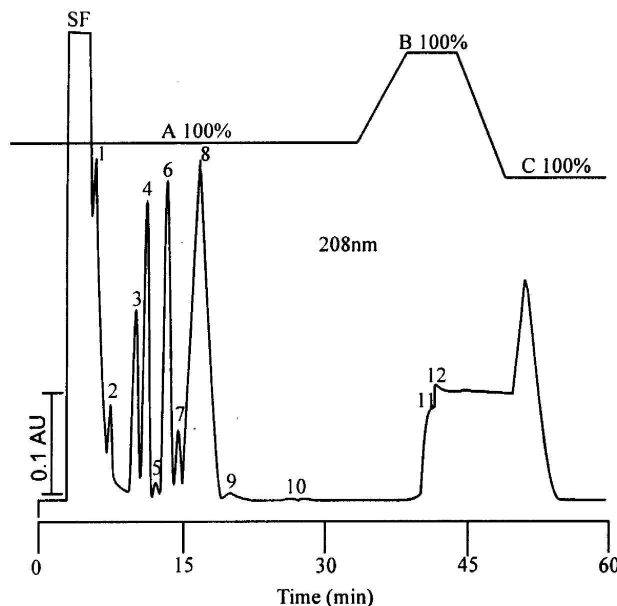


Figure 2. HPLC chromatograph of polar lipids from olive oil. Conditions and solvents A, B, and C in experimental section. Gradient as indicated. The numbers 1–12 indicate the HPLC fractions, which have been subjected to chemical analysis (see Table 2). SF: solvent front.

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

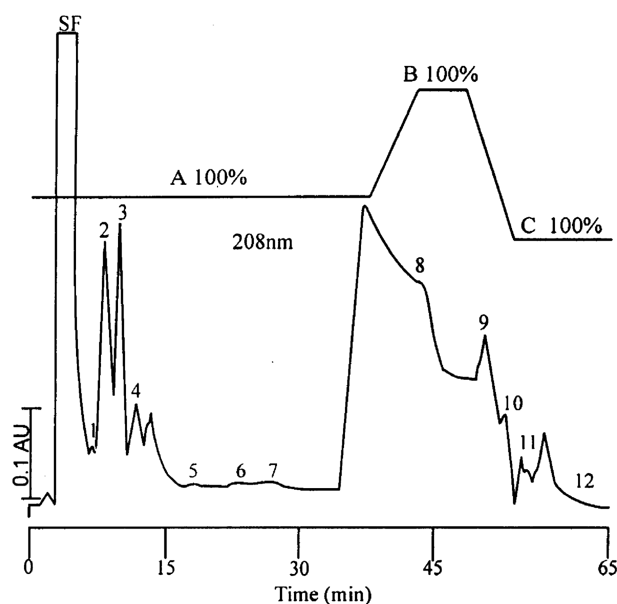


Figure 3. HPLC chromatograph of polar lipids from sunflower oil. Conditions and solvents A, B, and C in experimental section. Gradient as indicated. The numbers 1–12 indicate the HPLC fractions, which have been subjected to chemical analysis (see Table 3). SF: solvent front.

Phenolic determination was carried out with Folin-Ciocalteu reagent (33) as follows: A suitable aliquot of the fraction is transported into a test tube and dried under a stream of nitrogen. Afterwards, 3.5 mL of water and 0.1 mL of Folin Ciocalteu reagent are added. The content is mixed vigorously and after 3 min, 0.4 mL of saturated (ca. 35%) Na_2CO_3 solution is added. The content is again mixed vigorously and the extinction is measured after 1 hour at 725 nm, against a reagent black. Gallic acid served as a standard for preparing the calibration curve ranging from 0–40 μg/4 mL assay solution.

RESULTS

Separation of Standard Polar Lipids

Several mobile phase combinations were tested in order to achieve an efficient separation of lipid standards on a aminopropyl-modified silica gel HPLC column. Each individual standard was injected separately, and the resulting peak

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

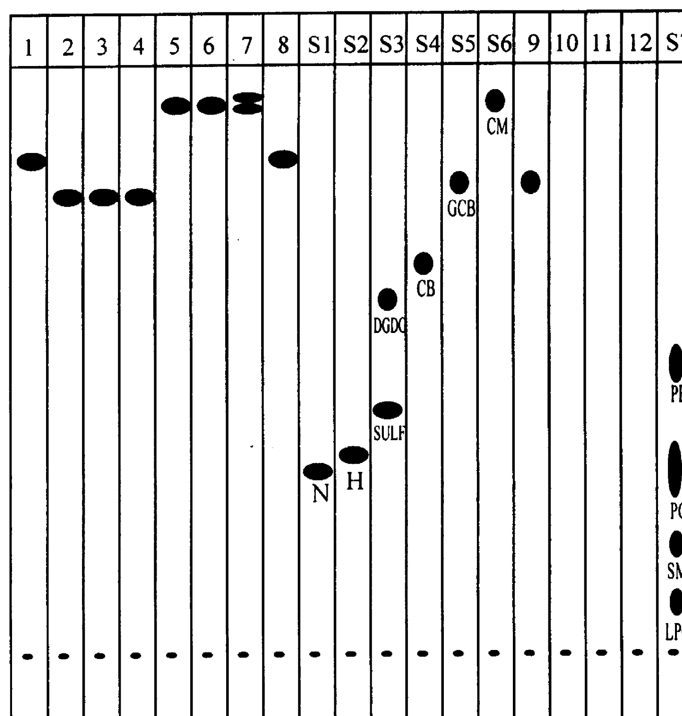


Figure 4. TLC development of standard lipids and of HPLC lipid fractions (1–12) from olive oil in chloroform/acetone/methanol/glacial acetic acid/water (50/20/17/5/5). S1: Naringin (N); S2: Hesperidin (H); S3: Sulfatides (SULF) and Digalactosyldiglycerides (DGDG); S4: Cerebrosides (CB); S5: Galactocerebrosides (GCB); S6: Ceramides (CM); S7: Phospholipids mixture: Lysophosphatidylcholine (LPC), Sphingomyelin (SM), Phosphatidylcholine (PC), Phospahtidyletanolamine (PE).

was collected and co-chromatographed on TLC plates with authentic standards in order to confirm their retention time (t_R). The final chosen solvent system consisted of an isocratic elution with 100% solvent A (acetonitrile-methanol: 70/30) for 35 min, followed by a linear gradient to 100% solvent B (methanol) in 5 min, a hold for 5 min in 100% B followed by a linear gradient to 100% solvent C (water) in 5 min, and, finally, a hold in 100% C for 10 min. For the next injection a 5 min elution with methanol and a 10 min equilibration with solvent A is required. The retention times of the injected standards, as well as a typical profile of their chromatographic separation are shown in Table 1 and in Fig. 1, respectively.

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Figure 5. TLC development of standard lipids and of HPLC lipid fractions (1–12) from sunflower oil in chloroform/acetone/methanol/glacial acetic acid/water (50/20/17/5/5). S1: Sulfatides (SULF); S2: Digalactosyldiglycerides (DGDG); S3: Galactocerebrosides (GCB); S4: Cerebrosides (CB); S5: Ceramides (CM); S6: Phospholipid mixture: Lysophosphatidylcholine (LPC), Sphingomyelin (SM), Phosphatidylcholine (PC), Phosphatidyletanolamine (PE).

Application to Olive Oil and Sunflower Oil Ethanol Extracts

Application of the described method for polar lipids of olive and sunflower oil are represented in Fig. 2 and 3, respectively. The results from the TLC development for the HPLC fractions from olive and sunflower oil are presented in Fig. 4 and 5. The results from chemical determinations for the above fractions are summarized in Table 2 and 3.

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Table 2. Chemical Determinations in HPLC Fractions from Polar Lipids of Olive Oil

HPLC Fractions	Glucose (µmol/mL)	Gallic Acid (µmol/mL)	Esters (µmol/mL)	Class
1	2.167	92.45	3.159	Glycophenolic
2	—	37.90	1.029	Phenolic
3	—	21.12	—	Phenolic
4	—	32.31	0.799	Phenolic
5	0.513	—	0.898	Glycolipid
6	1.751	9.930	—	Glycophenolic
7	—	—	—	—
8	2.631	11.33	—	Glycophenolic
9	0.462	—	—	Glycolipid
10	0.280	8.065	—	Glycophenolic
11	—	—	—	—
12	0.519	—	3.159	Glycolipid

Phosphorus determination was negative in all samples.

Table 3. Chemical Determinations in HPLC Fractions from Polar Lipids of Sunflower Oil

HPLC Fractions	Glucose (µmol/mL)	Gallic Acid (µmol/mL)	Esters (µmol/mL)	Class
1	—	—	—	—
2	0.677	—	—	Glycolipid
3	1.083	—	0.931	Glycolipid
4	—	—	—	—
5	—	0.147	—	Phospholipid
6	0.604	—	1.324	Glycolipid
7	—	—	—	—
8	1.187	—	—	Glycolipid
9	1.264	—	—	Glycolipid
10	0.568	—	—	Glycolipid
11	—	—	—	—
12	—	—	—	—

Phenolic determination was negative in all the samples.

DISCUSSION

It is well known that lipid compounds isolated from natural sources present significant biological activities. (1–2,34) This fact obviously requires separation systems to make possible the further study of these compounds and the

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

elucidation of their structures. Their identification may help us to understand the mechanisms for various pathophysiological conditions and the nutrition value of several food microconstituents.

Several methods for the HPLC separation of either phospholipids or glycolipids have previously been reported with various stationary phases. (35–38) Although, aminopropyl silica as a packing material has been used in many studies for the separation of lipid classes with solid phase extraction, (37–39) only a few studies have been made with aminopropyl modified silica based columns concerning HPLC separation of either phospholipids or glycolipids. (40–43) The most common use of this type of stationary phase has been for the HPLC separation of oligosaccharides. (44)

The method for the HPLC separation proposed here has the advantage of separating phospholipids, glycolipids, and phenolics, simultaneously, in a single run and in an efficient manner. In this way, the loss of microconstituents due to several experimental steps is minimized, so that it can be feasible to isolate compounds that exist in natural sources in minor amounts. The separation is completed in a reasonable time of approximately 1 h. The use of UV detection has low cost, easy operation, and is compatible with gradient elution. The aminopropyl silica is a moderately polar stationary phase, which would permit the elution of complex glycolipids with increased polarity that exist in natural sources and have significant biological activity. (1) In addition, the use of acid modifier for sharpening the peaks is omitted. This permits peak(s) to be collected, either for investigation of the structure and the possible biological activity, or for further fractionation by conventional methods suitable for each class.

The method seems to be applicable to polar lipids from olive oil and sunflower oil. The results from TLC analysis and chemical determination show that the compounds that have been separated from these two vegetable oils have structures different from the lipid standards used for the development of the HPLC system, and they only resemble polarity. This fact supports the existence of unknown minor constituents in polar lipid fraction of natural sources, which may have significant biological activity and nutrition value.

Downloaded At: 09:13 24 January 2011

REFERENCES

1. Fragopoulou, E.; Nomikos, T.; Antonopoulou, S.; Mitsopoulou, C.A.; Demopoulos, C.A. Separation of Biologically Active Lipids from Red Wine. J. Agric. Food Chem. **2000**, *48* (4), 1234–1238.
2. Rementzis, J.; Antonopoulou, S.; Argyropoulos, D.; Demopoulos, C.A. Biologically Active Lipids from S. Scombrus. Adv. Exp. Med. Biol. **1996**, *416*, 65–72.

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

3. Koussissis, S.; Semidalas, C.E.; Hadzistavrou, E.C.; Kalyvas, V.; Antonopoulou, S.; Demopoulos, C.A. PAF Antagonists in Food: Isolation and Identification of PAF Antagonists in Honey and Wax. Revue Francaise Des. Corps Gras **1994**, *5* (6), 127.
4. Koussissis, S.; Semidalas, C.E.; Antonopoulou, S.; Kapoulas, V.M.; Demopoulos, C.A.; Kalyvas, V. PAF Antagonists in Food: Isolation and Identification of PAF Antagonists in Virgin Olive Oil. Revue Francaise des Corp Gras. **1994**, *9* (10), 323.
5. Antonopoulou, S.; Semidalas, C.E.; Koussissis, S.; Demopoulos, C.A. Platelet-Activating Factor (PAF) Antagonists in Foods. A Study of Lipids, with PAF or Anti-PAF Like-Activity, in Cow's Milk and Yogurt. J. Agric. Food Chem. **1996**, *44*, 3047.
6. Rementzis, J.; Antonopoulou, S.; Demoipoulos, C.A. Identification and Study of Gangliosides from Scomber Scombrus Muscles. J. Agric. Food Chem. **1997**, *45*, 611.
7. Schieber, A.; Keller, P.; Carle, R. Determination of Phenolic Acids and Flavonoids of Apple and Pear by High-Performance Liquid Chromatography. J. Chromatogr. A. **2001**, *910* (2), 265–273.
8. Dawes, H.M.; Keene, J.B. Phenolic Composition of Kiwifruit Juice. J. Agric. Food Chem. **1999**, *47* (6), 2398–2403.
9. Kennedy, J.A.; Waterhouse, A.L.; Analysis of Pigmented High-Molecular-Mass Grape Phenolics Using Ion-Pair, Normal-Phase High-Performance Liquid Chromatography. J Chromatogr A **2000**, *866* (1), 25–34.
10. Kates, M. Techniques for Separation of Lipid Mixtures. In *Techniques of lipidology: Isolation, Analysis and Identification of Lipids*. 2nd Ed.; Work, T.S., Work, E., Eds.; Laboratory Techniques in Biochemistry and Molecular Biology; North-Holland: Amsterdam, 1975; Vol. 3 (Part II), 393–465.
11. Cremesti, A.E.; Fischl, A.S. Current Methods for the Identification and Quantitation of Ceramides: an Overview. Lipids. **2000**, *35* (9), 937–945.
12. Subbanagounder, G.; Watson, A.D.; Berliner, J.A. Bioactive Products of Phospholipid Oxidation: Isolation, Identification, Measurement and Activities. Free Radic. Biol. Med. **2000**, *28* (12), 1751–1761.
13. Abidi, S.L. Chromatographic Analysis of Tocol-Derived Lipid Antioxidants. J. Chromatogr. A **2000**, *881* (1–2), 197–216.
14. Patton, G.M.; Robins, S.J. Separation and Quantitation of Phospholipid Classes by HPLC. Methods Mol. Biol. **1998**, *110*, 193–215.
15. Cserhati, T.; Forgacs, E.; Morais, M.H.; Mota, T. Liquid Chromatography of Natural Pigments. Biomed. Chromatogr. **2000**, *14* (5), 281–286.
16. He, X.G. On-Line Identification of Phytochemical Constituents in Botanical Extracts by Combined High-Performance Liquid Chromatographic-Diode Array Detection-Mass Spectrometric Techniques. J. Chromatogr. A **2000**, *880* (1–2), 203–232.

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

17. Merken, H.M.; Beecher, G.R. Measurement of Food Flavonoids by High-Performance Liquid Chromatography: A review. J. Agric. Food Chem. **2000**, *48* (3), 577–599.
18. Beecher, G.R.; Warden, B.A.; Merken, H. Analysis of Tea Polyphenols. Proc. Soc. Exp. Biol. Med. **1999**, *220* (4), 267–270.
19. Khurana, A.L. Food Analysis on Silica-Bound HPLC Phases. Crit. Rev. Food Sci. Nutr. **1990**, *29* (3), 197–235.
20. Verhaar, L.A.; Kuster, B.F. Liquid Chromatography of Sugars on Silica-Based Stationary Phases. J. Chromatogr. **1981**, *220* (3), 313–328.
21. Abbott, S.R.; Practical Aspects of Normal-Phase Chromatography. J. Chromatogr. Sci. **1980**, *18* (10), 540–550.
22. Christie, W.W. Rapid Separation and Quantification of Lipid Classes by High Performance Liquid Chromatography and Mass (light-scattering) Detection. J. Lipid Res. **1985**, *26* (4), 507–12.
23. Hamilton, J.G.; Comai, K. Separation of Neutral Lipid, Free Fatty Acid and Phospholipid Classes by Normal Phase HPLC. Lipids **1988**, *23* (12), 1150–1153.
24. Boselli, E.; Grob, K.; Lercker, G. Determination of Furan Fatty Acids in Extra Virgin Olive Oil. J. Agric. Food Chem. **2000**, *48* (7), 2868–2873.
25. Chen, S.H.; Chen, K.C.; Lien, H.M. Determination of Fatty Acids in Vegetable Oil by Reversed-Phase Liquid Chromatography with Fluorescence Detection. J. Chromatogr. A **1999**, *849* (2), 357–369.
26. Feeter, D.K. Determination of Tocopherols, Sterols, and Steryl Esters in Vegetable Oil Distillates and Residues. J. Am. Oil Chem. Soc. **1974**, *51* (4), 184–187.
27. Galanos, D.S.; Kapoulas, V.M. J. Lipid. Res. **1962**, *3*, 134.
28. Rouser, G.; Kritchevsky, G.; Yamamoto, A. Column Chromatographic and Associated Procedures for Separation and Determination of Phosphatides and Glycolipids. In *Lipid Chromatographic Analysis*, 2nd Ed.; Marinetti, G.V., Ed.; Marcel Dekker, Inc.: New York, 1976; Vol. 3, 713–776.
29. Siakotos, A.N. Analytical Separation of Nonlipid Water Soluble Substances and Gangliosides from Other Lipids by Dextran Gel Column Chromatography. Am. Oil Chem. Soc. **1965**, *42* (11), 913–919.
30. Bartlett, G. R. Phosphorus Assay in Column Chromatography. J. Biol. Chem. **1959**, *234*, 466–468.
31. Renkonen, O. A Note on Spectrophotometric Determination of Ester Groups in Lipids. Biochim. Biophys. Acta **1961**, *54*, 361.
32. Galanos, D. S.; Kapoulas, V. M. Preparation and Analysis of Lipid Extracts from Milk and Other Tissues. Biochim. Biophys. Acta **1965**, *98*, 278–292.
33. Swain, T.; Hillis, W.E. J. Sci. Food Agric. **1959**, *10*, 63.
34. Demopoulos, C.A.; Antonopoulou, S. A Discovery Trip to Compounds with PAF-Like Activity. Adv. Exp. Med. Biol. **1996**, *416*, 59–63.

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

35. McCluer, R.H.; Ullman, M.D.; Jungalwala, F.B. HPLC of Glycosphingolipids and Phospholipids. Adv. Chromatogr. **1986**, *25*, 309–353.
36. Demopoulos, C.A.; Kyrili, M.; Antonopoulou, S.; Andrikopoulos, N.K. J. Liq. Chrom. & Rel. Technol. **1986**, *19* (5), 771–781.
37. Andrikopoulos, N.K.; Demopoulos, C.A.; Siafaka-Kapadai, A. High-Performance Liquid Chromatographic Analysis of Platelet Activating Factor on a Cation-Exchange Column by Direct Ultraviolet Detection. J. Chromatogr. **1986**, *363* (2), 412–417.
38. Ruiz-Gutierrez, V.; Perez-Camino, M.C. Update on Solid-Phase Extraction for the Analysis of Lipid Classes and Related Compounds. J. Chromatogr. A **2000**, *885* (1–2), 321–341.
39. Suzuki, E.; Sano, A.; Kuriki, T.; Miki, T. Improved Separation and Determination of Phospholipids in Animal Tissues Employing Solid Phase Extraction. Biol. Pharm. Bull. **1997**, *20* (4), 299–303.
40. Kim, H.Y.; Salem, N. Jr. Separation of Lipid Classes by Solid Phase Extraction. J. Lipid Res. **1990**, *31* (12), 2285–2289.
41. Wada, M.; Nakashima, K.; Kuroda, N.; Akiyama, S.; Imai, K. Sensitive Flow-Injection Method With Peroxyoxalate Chemiluminescence Detection Combined with Preparative High-Performance Liquid Chromatography for Determination of Choline-Containing Phospholipids in Human Serum. J. Chromatogr. B Biomed. Appl. **1996**, *678* (2), 129–136.
42. Hanson, V.L.; Park, J.Y.; Osborn, T.W.; Kiral, R.M. High-Performance Liquid Chromatographic Analysis of Egg Yolk Phospholipids. J. Chromatogr. **1981**, *205* (2), 393–400.
43. Bernhard, W.; Linck, M.; Creutzburg, H.; Postle, A.D.; Arning, A.; Martin-Carrera, I.; Sewing, K.F. High-performance Liquid Chromatographic Analysis of Phospholipids from Different Sources with Combined Fluorescence and Ultraviolet Detection. Anal. Biochem. **1994**, *220* (1), 172–180.
44. Turco, S.J. Rapid Separation of High-Mannose-Type Oligosaccharides by High-Pressure Liquid Chromatography. Anal. Biochem. **1981**, *118* (2), 278–283.

Received July 18, 2001
Accepted August 12, 2001
Manuscript 5617

Downloaded At: 09:13 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Downloaded At: 09:13 24 January 2011

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081JLC100108545