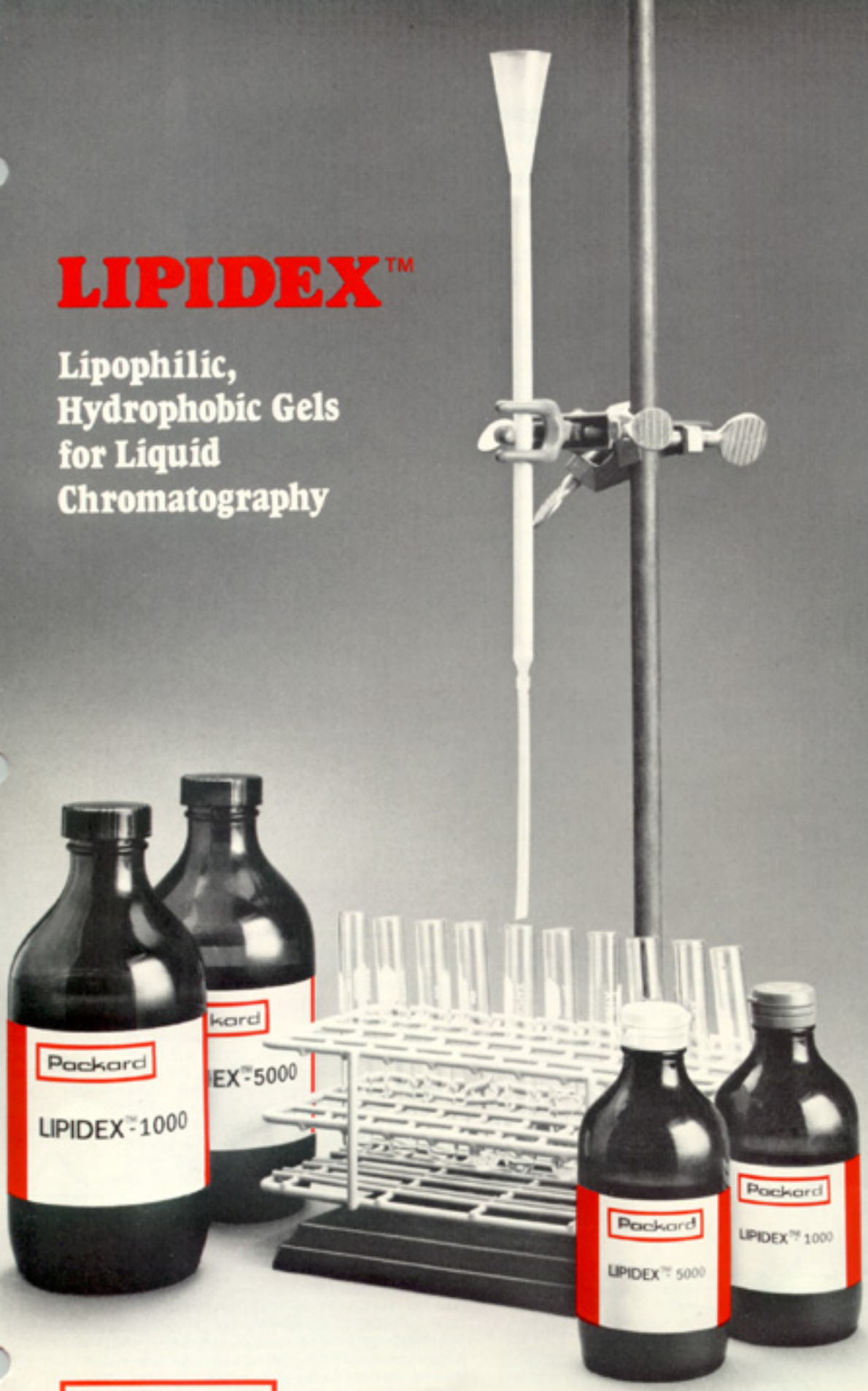
LIPIDEX™

Lipophilic, Hydrophobic Gels for Liquid Chromatography



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WHAT IS LIPIDEX?

LIPIDEX is a column packing material for liquid chromatography used to separate a variety of steroids, prostaglandins, lipids, and numerous other natural products. LIPIDEX is a major advance in the development of column packings used in the separation and purification of lipid substances.

Since the introduction in 1959 of gel filtration in aqueous media[1], there have been many developments in column packings. Among the early developments were the Sephadex* G-type gels which swell in very polar solvents and in water. These G-type gels are useful for the separation of proteins, polysaccharides, enzymes, polynucleotides, peptides, hormones, amino acids and other materials by gel filtration. Soon after the G-type gels were introduced, gels which would swell in nonpolar solvents were developed for similar separations[2]. These gels, however, are of limited interest to the biochemist working in the fields of lipid and steroid chemistry or chemists working in other areas of natural product chemistry[3].

In 1966, Pharmacia Fine Chemicals introduced Sephadex LH-20, a hydroxypropyl derivative of Sephadex G-25[3]. While answering many of the needs for a lipophilic gel, Sephadex LH-20 is polar and not hydrophobic, and thus cannot be used for reversed phase chromatography.

The search for a hydrophobic derivative of Sephadex was continued for at least two reasons: 1) nonpolar solvents offer a milder environment for the sample and usually can be removed from the sample under milder conditions, and 2) nonpolar lipids are best separated by reversed phase chromatography based on nonpolar interactions with the stationary support. In 1968 such a derivative was developed[4]. This derivative is a hydroxyalkoxypropylation product of Sephadex, and the long alkyl chains covalently bound to the polysaccharide matrix give the gel beads the solvent properties of an insoluble hydrophobic lipid.

PACKARD offers two such lipophilic, hydrophobic derivatives: LIPIDEX-1000 and LIPIDEX-5000. Both preparations are alkoxy group derivatives of Sephadex LH-20. The average alkoxy group chain length is 15 carbons. LIPIDEX-1000 is approximately 10% substituted, and LIPIDEX-5000 is approximately 50% substituted.

*Registered T.M. of Pharmacia Fine Chemicals, AB Uppsala, Sweden

ORDERING INFORMATION

	CATALOG NUMBER	PACKAGE SIZE
LIPIDEX-1000	6008301	25 g
	6008302	100 g
LIPIDEX-5000	6008303	25 g
	6008304	100 g

APPLICATIONS

Hydroxyalkoxypropyl derivatives of Sephadex have demonstrated advantages in many types of preparations. LIPIDEX-1000 and LIPIDEX-5000 have been formulated to meet the requirements of these separations.

PROSTAGLANDINS

Prostaglandins constitute a group of closely related lipids with important biological properties. A hydroxyalkoxypropyl derivative of Sephadex containing 11-12% hydroxyalkyl groups (similar to LIPIDEX-1000) has been used for separations of prostaglandins A, B, C, E, and F by reversed phase partition chromatography[2-4]. Methyl esters of prostaglandins E and F have also been separated using these materials. *(See Figure 1 and Figure 2.)*

The reversed phase separation of prostaglandins on LIPIDEX-1000 offers several advantages:

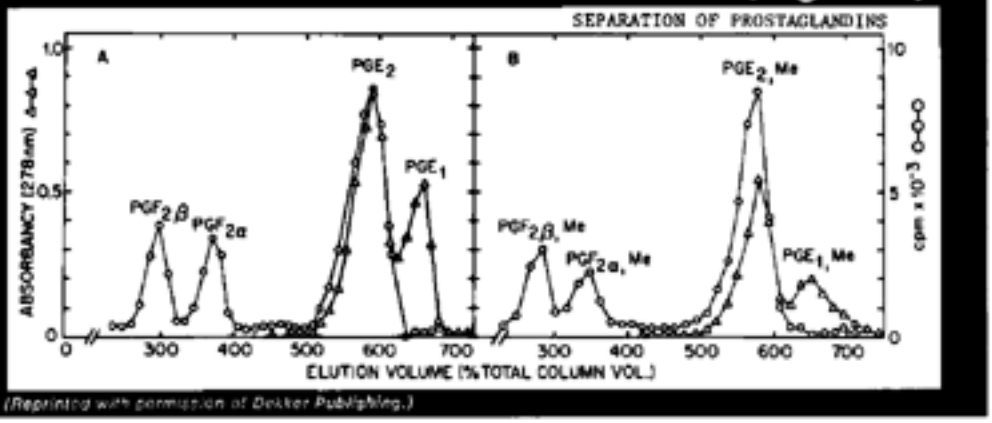
- Greater solubility of some prostaglandins in mobile phase used with LIPIDEX-1000[4].
- Better separation of prostaglandins B_1 and C_1[5].
- Large sample loads on columns without serious peak broadening[5].
- Prepared columns can be used for several months without deterioration[4].

Separations of methyl esters of prostaglandins have also been accomplished by straight phase separation on LIPIDEX-5000. *(See Figure 3 and Figure 4.)*

RADIOIMMUNOASSAY OF STEROIDS

With effective purification procedures prior to radioimmunoassays, antisera with less specificity are required. A 50% substituted derivative, similar to LIPIDEX-5000, separates several unconjugated neutral steroids present in human plasma[4]. This type of material has been used to isolate purified fractions of testosterone, progesterone, and 17α-hydroxyprogesterone from human plasma prior to radioimmunoassay[6]. *(See Figure 5.)*

(Figure 1)[3]



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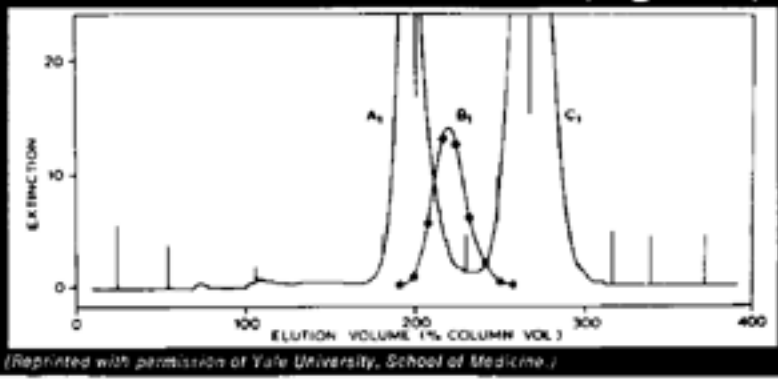
A. Separation of ^{3}H-PGF$_2\beta$ (50 ng), ^{3}H-PGF$_2\alpha$ (50 ng), ^{3}H-PGE$_2$ (50 μg) and PGE$_1$ (50 μg). The sample was applied in 1 ml of the eluting solvent containing 50 μl of acetic acid. Column: 235 x 10 mm, hydrophobic Sephadex, 140-170 mesh, hydroxyalkyl content 12%. Solvent system water/methanol/*n*-butanol/chloroform, 60:40:7:3 (by vol.). Flow rate 10 ml/h. Temperature 25°.

B. Separation of the methyl esters of the same prostaglandins as those shown in A. The sample was dissolved in 1 ml of the eluting solvent: water/methanol/*n*-butanol/chloroform, 50:50:5:5 (by vol.), and applied to a column, 210 x 10 mm. Other chromatographic conditions were as in A.

(Figure 2)[6]



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Chromatography of PGA$_1$, B$_1$ and C$_1$ on a column (10 x 350 mm) of LIPIDEX 1000 gel. Solvent system: water/methanol/*n*-butanol/chloroform 40:60:4:6 (by vol.) and 0.1% acetic acid. Flow rate: 6.5 ml/h. PGA$_1$ and C$_1$ were detected by continuous monitoring of the column effluent for light extinction at 242 nm. PGB$_1$ (⟷) was estimated in individual fractions from the intensity of its extinction maximum at 280 nm. ^{3}H-PGB$_2$ (0.5 ng) was also included and was eluted at 205% of the column volume.

Similarly, testosterone has been separated from 5α-dihydrotestosterone. This separation provides a method of isolating purified testosterone free of 5α-dihydrotestosterone which interferes with the radioimmunoassay of testosterone. *(See Figure 6.)*

In the absence of an antiserum of sufficient specificity to quantify the steroid of interest, purification of the sample is required prior to the RIA. LIPIDEX may be effective for the purification of many steroids for radioimmunoassay.

GAS CHROMATOGRAPHY-MASS SPECTROMETRIC ANALYSIS OF STEROIDS

LIPIDEX-5000 and derivatives similar to LIPIDEX-5000 have been used to separate groups of steroids prior to gas chromatographic-mass spectrometric analysis[10,11,12]. By reversed phase chromatography on LIPIDEX-5000, nonpolar lipids can be removed from tissue extracts, and further purification is accomplished by straight phase chromatography on LIPIDEX-5000[10]. Similarly, urinary steroid extracts can be chromatographed using straight phase and reversed phase solvent systems[12]. Both straight phase and reversed phase systems on LIPIDEX-5000 are useful as preliminary separation methods for gas chromatography or gas chromatography-mass spectrometric analysis of steroids.

OTHER APPLICATIONS

Preparations similar to LIPIDEX-1000 and LIPIDEX-5000 have been used in a wide variety of applications. A number of related references are provided in the Bibliography.

PROPERTIES OF LIPIDEX

SWELL CHARACTERISTICS OF LIPIDEX AT 20°C.

SOLVENTS	*MILLILITERS PER GRAM*	
	LIPIDEX-1000	LIPIDEX-5000
Methanol	3.8	2.5
Isopropanol	3.6	3.0
n-Pentyl alcohol	3.7	3.4
Water/Methanol/1-Butanol/Chloroform (60/40/7/3)	3.6	3.7
Water/Isopropanol/Carbon tetrachloride (30/60/17)	4.6	3.9
Chloroform/Methanol (2/1)	4.9	4.1
Carbon tetrachloride	2.3	4.0
n-Hexane	1.9	3.2
Acetone	3.0	2.7
Cyclohexane	2.1	3.5
Benzene	2.7	4.3
Toluene	2.5	4.4

POLARITY

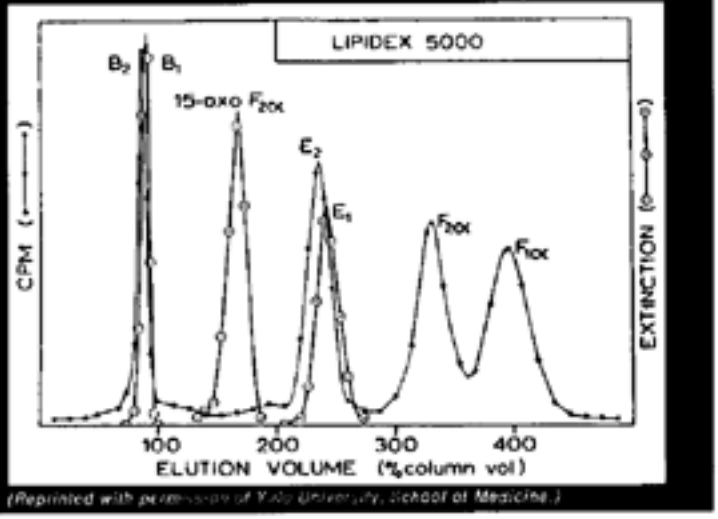
The polarity of LIPIDEX is a function of the degree of alkoxylation. As the amount of alkoxylation is increased, the polarity is decreased. LIPIDEX-1000 is more polar than LIPIDEX-5000.

STABILITY

LIPIDEX is provided in a methanol suspension to prolong its shelf life. In methanol, the shelf life of LIPIDEX is at least two years.

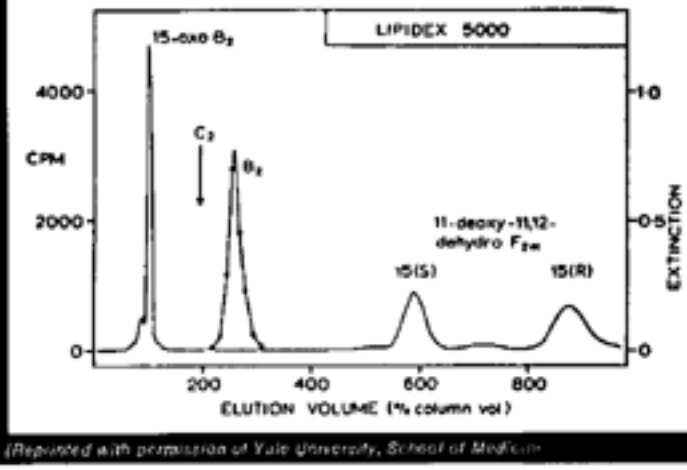
LIPIDEX can be used with a number of solvent systems. Care should be taken to avoid the use of LIPIDEX with strongly acidic solutions or solutions that contain oxidizing agents.

(Figure 3)[7]



Separation of the methyl esters of PGB_1 (50 μg), ^{3}H-PGB_2 (1 ng), PGE_1 (100 μg), ^{3}H-PGE_2 (1 ng), 15-oxo $PGF_2\alpha$ (1 mg), ^{3}H-$PGF_1\alpha$ (1 ng) and ^{3}H-$PGF_2\alpha$ (1 ng) on LIPIDEX 5000-70:30 (heptane-chloroform v/v). Column: 9 x 390 mm. Flow rate: 15 ml/h. Temperature: 23°C.

(Figure 4)



Separation of the methyl esters of 15-oxo PGB_2 (150 μg), ^{3}H-PGB_2 (1 μg), PGC_2, and 15(S)- and 15(R)-11-deoxy-11,12-dehydro $PGF_2\alpha$ (400μg each) on LIPIDEX 5000-90:10 (heptane-chloroform v/v). The elution volume of PGC_2 methyl ester was determined from another run on the same column. Column: 10 x 352 mm. Flow rate: 13 ml/h. Temperature: 23°C.

PREPARATION OF LIPIDEX COLUMNS

1) Transfer a suitable amount of the methanolic suspension of LIPIDEX to a Büchner funnel or a centrifuge tube. Remove the methanol by suction or centrifugation and wash the gel with about four volumes of the solvent in which the gel is to be equilibrated.

2) Slurry the gel volume in at least five volumes of the solvent to be used for chromatography. Stir in an ultrasonic bath to obtain a slurry free from lumps of adhering gel beads and air bubbles. Leave for equilibration. The time required depends on the nature of the solvents and may be very short. Often it is advisable to leave the slurry for 1-2 hours before packing the column.

When reversed phase solvents with high water content are used, equilibration is slower. When the water content exceeds 30% (e.g., methanol/water/chloroform/1-butanol, 50/50/5/5), LIPIDEX should be left to swell in the solvent components first without water. After equilibration with the solvent mixture, the water is added and equilibration is continued for an additional two hours.

Pour the gel slurry into a column partially filled with the solvent. The column is packed by gravity flow. Packing is more uniform if the column is rotated between the hands at intervals.

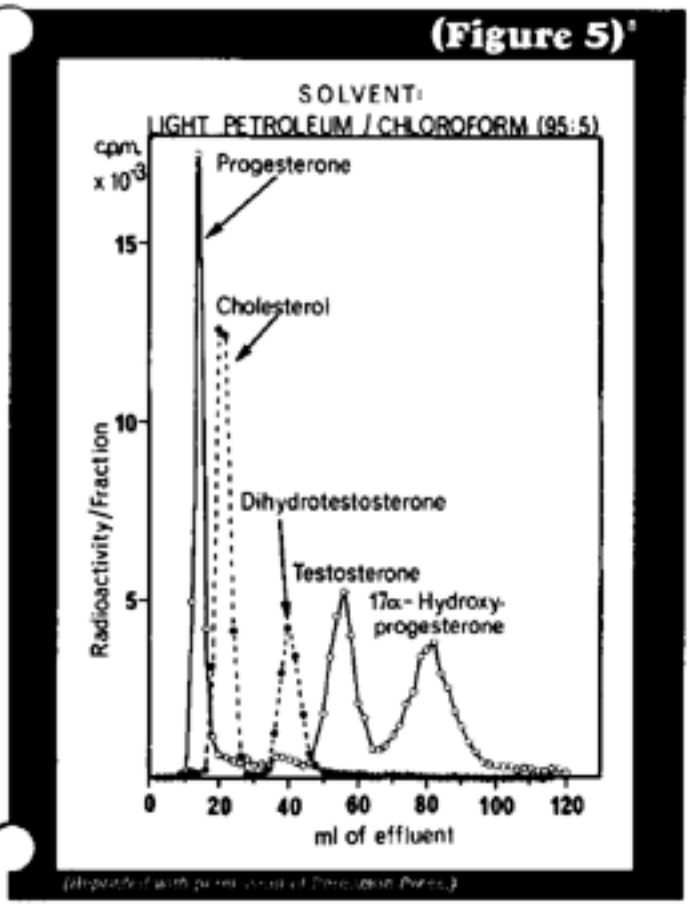
Capillary columns (e.g., in Teflon** tubing 1000 x 1.5 mm) are filled from a reservoir attached to the top of the column containing the slurry of LIPIDEX. The column is first filled completely with the solvent mixture. Then the reservoir containing the gel slurry is attached and a pressure of about 1 atmosphere from a nitrogen tank is applied to the reservoir. The reservoir should be vibrated continuously.

Depending on the solvent composition, a column can be used for several months. When not used, the column should be left under solvent. Chlorinated solvents will slowly degrade the support.

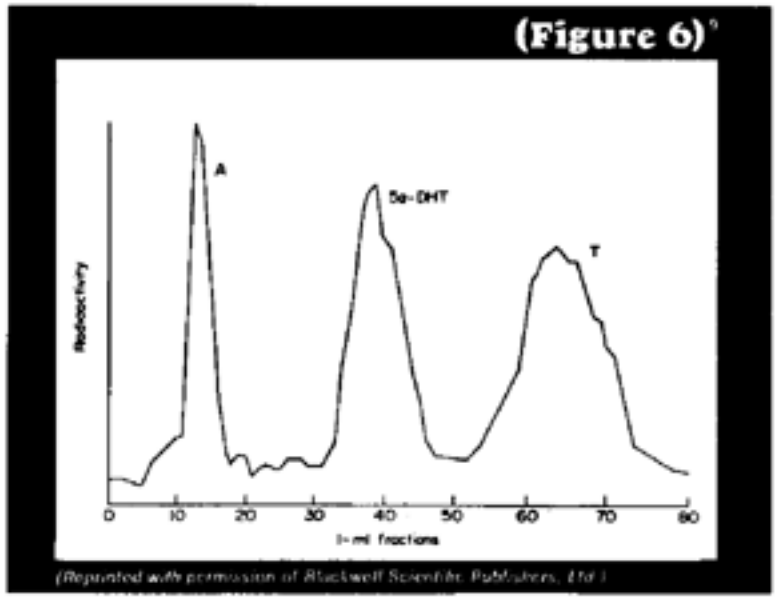
** Registered T.M. of E. I. DuPont de Nemours & Co.

FOOTNOTES

1. J. Porath and P. Flodin, *Nature, 183*, 1657-1659 (1959).
2. J. C. Moore, *Journal of Polymer Science*, Part A2, 835-843 (1964).
3. *Sephadex-LH-20*, (product brochure) Pharmacia Fine Chemicals AB, October, 1970.
4. J. Ellingboe, E. Nyström and J. Sjövall, *Biochimica et Biophysica Acta, 152*, 803 (1968).
5. E. Nyström and J. Sjövall, *Analytical Letters, 6*(2), 155-161, (1973).
6. R. L. Jones, *Prostaglandins*, 5(3), 283-290 (1974).
7. A. R. Brash and R. L. Jones, *Prostaglandins, 10*, 441-454 (1974).
8. O. Jänne, D. Apter and R. Vihko, *Journal of Steroid Biochemistry, 5*, 155-162 (1974).
9. J. P. P. Tyler, *Journal of Reproduction and Fertility, 33*, 357-359 (1973).
10. M. Axelson, G. Schumacher and J. Sjövall, *Journal of Chromatographic Science, 12*, 535-540 (1974).
11. W. E. Brazelton, Jr., J. C. Orr and L. L. Engel, *Analytical Biochemistry, 53*, 64-65 (1973).
12. R. A. Anderson, G. Defaye, C. Madani, E. M. Chambaz and C. J. W. Brooks, *Journal of Chromatography, 99*, 485-494 (1974).



Elution of certain steroids from a 2.5 g column of hydroxyalkoxypropyl Sephadex in light petroleum chloroform, 95:5.



Separation of androgens on hydroxy-alkoxy-propyl Sephadex.

BIBLIOGRAPHY

SURVEY AND METHODOLOGICAL ARTICLES

C. J. W. Brooks and R. A. B. Keates, "Gel Filtration in Lipophilic Solvents using Hydroxyalkoxypropyl Derivatives of Sephadex," *Journal of Chromatography, 44,* 509-521 (1969).

J. Ellingboe, E. Nyström and J. Sjövall, "A Versatile Lipophilic Sephadex Derivative for Reversed-phase Chromatography," *Biochimica et Biophysica Acta, 152,* 803 (1968).

J. Ellingboe, E. Nyström and J. Sjövall, "Chromatography on Lipophilic Sephadex," *Methods in Enzymology,* Vol. 14, Lipids, J. M. Lowenstein (ed.), Academic Press, New York, 1969, pp. 317-329.

E. Nyström and J. Sjövall, "Chromatography on Lipophilic Sephadex," *Methods in Enzymology,* Vol. 35, Lipids, J. M. Lowenstein (ed.), Academic Press, New York, 1974, in press.

PROSTAGLANDINS

A. R. Brash and R. L. Jones, "Straight Phase Separation of Prostaglandin Methyl Esters on Lipophilic Gels," *Prostaglandins, 10,* 441-454 (1974). (LIPIDEX-1000 and LIPIDEX-5000)

R. L. Jones, "Preparation of Prostaglandins C: Chemical Fixation of Prostaglandin A Isomerase to a Gel Support and Partition Chromatography of Prostaglandins A, B and C," *Prostaglandins, 5* (3), 283-290 (1974). (LIPIDEX-1000)

E. Nyström and J. Sjövall, "Separation of Prostaglandins on a Hydrophobic Sephadex Derivative," *Analytical Letters, 6,* 155 (1973). (Derivative similar to LIPIDEX-1000 for separation of Prostaglandins E and F)

STEROIDS

R. A. Anderson, G. Defaye, C. Madani, E. M. Chambaz and C. J. W. Brooks, "Lipophilic Gel and Gas-Phase Analysis of Steroid Hormones," *Journal of Chromatography, 99,* 485-494 (1974). (Derivative similar to LIPIDEX-5000 used for separations of urinary steroid extracts prior to gas chromatography or gas chromatography-mass spectrometric analysis)

M. Axelson, G. Schumacher and J. Sjövall, "Analysis of Tissue Steroids by Liquid-Gel Chromatography and Computerized Gas Chromatography-Mass Spectrometry," *Journal of Chromatographic Science, 12,* 535 (1974). (LIPIDEX-5000 used for group purification of tissue steroids prior to gas chromatographic-mass spectrometric analysis)

M. Axelson and J. Sjövall, "Separation and Computerized Gas Chromatography-Mass Spectrometry of Unconjugated Neutral Steroids in Plasma," *Journal of Steroid Biochemistry, 5* (1974), in press. (Derivative similar to LIPIDEX-5000 used for group purification of neutral steroids in plasma and for purification of steroid trimethylsilyl ethers prior to gas chromatographic-mass spectrometric analysis)

W. E. Braselton, Jr., J. C. Orr, and L. L. Engle, "The Twin Ion Technique for Detection of Metabolites by Gas Chromatography-Mass Spectrometry: Intermediates in Estrogen Biosynthesis," *Analytical Biochemistry, 53,* 64 (1973). (Derivative similar to LIPIDEX-5000 used for purification of steroids)

J. Ellingboe, E. Nyström and J. Sjövall, "Liquid-Gel Chromatography on Lipophilic-Hydrophobic Sephadex Derivatives," *Journal of Lipid Research, 11,* 266-273 (1970). (Separations of many different types of samples including nonpolar steroids, and progestins and androgens on derivative similar to LIPIDEX-5000)

T. Holmdahl and J. Sjövall, "Liquid-Gel Chromatography on Hydrophobic Sephadex and Competitive Protein Binding of 17α-Hydroxyprogesterone in Plasma," *Steroids, 18,1,* 69-76 (1971). (Separation of androgens and progestin on derivative similar to LIPIDEX-5000. Purification of 17α-Hydroxyprogesterone for RIA.)

O. Jänne, D. Apter and R. Vihko, "Assay of Testosterone, Progesterone and 17α-Hydroxyprogesterone in Human Plasma by Radioimmunoassay after Separation on Hydroxyalkoxypropyl Sephadex," *Journal of Steroid Biochemistry, 5,* 155-162 (1974). (Derivative similar to LIPIDEX-5000)

K. Kontula, O. Jänne, T. Luukkainen and R. Vihko, "Progesterone — Binding Protein in Human Myometrium," *Biochimica et Biophysica Acta, 328,* 145-153 (1973). (Derivative similar to LIPIDEX-5000 used to purify steroids prior to use)

A. Ruokonen, "Free and Sulphate-Conjugated 16-Unsaturated C_{19} Steroids in Human Testis Tissue," *Biochimica et Biophysica Acta, 316,* 251-255 (1973). (Derivative similar to LIPIDEX-5000 used to purify steroids)

J. P. P. Tyler, "Problems with a Radioimmunoassay for Testosterone," *Journal of Reproduction and Fertility, 33,* 357-359 (1973). (Separation of testosterone, dihydrotestosterone and androsterone on derivative similar to LIPIDEX-5000)

NEUTRAL LIPIDS

L. Aringer and P. Eneroth, "Studies on the Formation of C_7-Oxygenated Cholesterol and β-Sitosterol Metabolites in Cell-Free Preparations of Rat Liver," *Journal of Lipid Research, 14,* 563 (1973). (Cholesterol, β-Sitosterol and their metabolites separated on derivative similar to LIPIDEX-5000)

C. J. W. Brooks and R. A. B. Keates, "Gel Filtration in Lipophilic Solvents using Hydroxyalkoxypropyl Derivatives of Sephadex," *Journal of Chromatography, 44,* 509-521 (1969). (Purification and group separation of neutral and polar lipids using derivative similar to LIPIDEX-5000)

T. Curstedt and J. Sjövall, "Analysis of Molecular Species of ^{2}H-Labelled Phosphatidylcholines by Liquid-Gel Chromatography and Gas Chromatography-Mass Spectrometry," *Biochimica et Biophysica Acta, 360,* 24-37 (1974). (Derivative similar to LIPIDEX-5000 used for reversed phase chromatography of trimethylsilyl ethers of diglycerides)

J. Ellingboe, E. Nyström and J. Sjövall, "Liquid Gel Chromatography on Lipophilic-Hydrophobic Sephadex Derivatives," *Journal of Lipid Research, 11,* 266 (1970). (Separations of long chain waxes, cholesteryl esters, monoglycerides and triglycerides using derivative similar to LIPIDEX-5000)

J. Ellingboe and D. Steinberg, "Differential Susceptibility of Phytanyl and Palmityl Ester Bonds to Enzymatic Hydrolysis," *Biochimica et Biophysica Acta, 270,* 92-102 (1972). (Separation and purification of α,α'-digylcerides and α,β-diglycerides on derivative similar to LIPIDEX-5000)

P. M. Hyde and W. H. Elliott, "Separation of β-Sitosterol and Campesterol on Hydrophobic Hydroxyalkyl Sephadex LH-20," *Journal of Chromatography, 67,* 170 (1972). (Derivative similar to LIPIDEX-5000)

B. Lindqvist, I. Sjögren and R. Nordin, "Preparative Fractionation of Triglyceride Mixtures According to Acyl Carbon Number using Hydroxyalkoxypropyl Sephadex," *Journal of Lipid Research, 15,* 65-73 (1974). (Derivative similar to LIPIDEX-5000)

FATTY ACIDS AND BILE ACIDS

K. Beijer and E. Nyström, "Reversed-phase Chromatography of Fatty Acids on Hydrophobic Sephadex," *Analytical Biochemistry, 48,* 1-8 (1972). (Derivative similar to LIPIDEX-5000)

T. Cronholm, I. Makino and J. Sjövall, "Steroid Metabolism in Rats," *European Journal of Biochemistry, 24,* 507-519 (1972). (Separation of bile acids on derivative similar to LIPIDEX-5000)

J. Ellingboe, E. Nyström and J. Sjövall, "Liquid Gel Chromatography on Lipophilic-Hydrophobic Sephadex Derivatives," *Journal of Lipid Research, 11,* 266 (1970). (Fatty acids and bile acids separated on derivative similar to LIPIDEX-5000)

OTHER COMPOUNDS

L. Ahlquist, B. Olsson, A. Ståhl and J. Ställberg-Stenhagen, "Studies on Natural Odoriferous Compounds," *Chimica Scripta, 1,* 237-246 (1971). (Separation of labile terpenoid compounds on derivative similar to LIPIDEX-5000)

C. J. W. Brooks and R. A. B. Keates, "Gel Filtration in Lipophilic Solvents using Hydroxyalkoxypropyl Derivatives of Sephadex," *Journal of Chromatography, 44,* 509 (1969). (Separation of terpenoid compounds on derivative similar to LIPIDEX-5000)

G. J. Krol, C. A. Mannon, F. Q. Gemmill, Jr., G. E. Hicks and B. T. Kho, "High-Efficiency Liquid Chromatographic Separation of Vitamin D from Precalciferol," *Journal of Chromatography, 74,* 43-49 (1972). (Derivative similar to LIPIDEX-5000 for preparative separation and purification of Vitamin D from a large excess of Vitamin A)

D. S. Stephenson and C. J. W. Brooks, "Separation and Characterization of Δ^3- and Δ^4-Enclopyrethrosen Acetates via Chromatography on a Lipophilic Dextran Gel," *Journal of Chromatography, 75,* 308 (1973). (Analytical and preparative separation of terpenoid isomers on derivative similar to LIPIDEX-5000)

J. N. Thompson, P. Erdody and W. B. Maxwell, "Chromatographic Separation and Spectrophotometric Determination of Tocopherols using Hydroxyalkoxpropyl Sephadex," *Analytical Biochemistry, 50,* 267 (1972). (Derivative similar to LIPIDEX-5000)

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