1.

2.

Storage of tissues

The solubility of lipids in organic solvents

3. Removal of non-lipid contaminants4. Artefacts of extraction procedures

Some practical considerations

Contents

Summary		xiii	
1.	The	Structure, Chemistry and Occurrence of Lipids	1
•	<i>A</i> .	Introduction	1
	В.	The Fatty Acids	
- es		 Saturated fatty acids Monoenoic fatty acids Non-conjugated polyunsaturated fatty acids Branched-chain and cyclopropane fatty acids Some unusual fatty acids of plant and animal origin 	1 2 3 5 6
	<i>C</i> .	Simple Lipids 1. Triacylglycerols and partial glycerides 2. Alkyl-diacylglycerols and neutral plasmalogens 3. Cholesterol and cholesterol esters 4. Wax esters and other simple lipids	7 7 8 9 9
	D.	Complex Lipids 1. Glycerophospholipids 2. Glycoglycerolipids and related compounds 3. Sphingolipids	10 12 13
	E.	Structural Features of Lipids Important in Analyses	15
2.	The	e Isolation of Lipids from Tissues	17
	Α.	General Principles of Solvent Extraction Procedures	17

17

17 19

20 21

viii		Contents	
	В.	Recommended Procedures	21
		 Extraction of large amounts of tissue Chloroform-methanol (2:1, v/v) extraction and "Folch" wash Sephadex G-25 columns for removing non-lipid contaminants 	22 22 23
3,	Chro	omatographic and Spectroscopic Analysis of Lipids. General Principles.	25
l 5.	A.	A Statement of the Problem	25
	В.	Chromatographic Procedures	26
	وت.	 Gas-liquid chromatography Adsorption column chromatography Thin-layer adsorption chromatography Liquid-liquid partition chromatography Chromatography on adsorbents containing complexing agents Ion-exchange cellulose chromatography 	26 30 34 38 40 42
	<i>C</i> .	Spectroscopy	42
	жет - 34	 Infrared absorption spectroscopy Ultraviolet absorption spectroscopy Nuclear magnetic resonance spectroscopy Mass spectrometry Optical rotary dispersion 	43 44 44 45 46
İ	D.	Some practical considerations	46
		 Autoxidation of lipids Solvents Contaminants Equipping a laboratory for lipid analysis 	46 47 48 48
4.	The	Preparation of Derivatives of Lipids	51
	A.	Hydrolysis (saponification) of Lipids	51
1	B.	The Preparation of Methyl and other Esters of Fatty Acids	52
		 Acid-catalysed esterification and transesterification Base-catalysed transesterification Diazomethane Special cases Preparation of esters other than methyl 	52 53 54 55 56
	<i>C</i> .	Derivatives of Hydroxyl Groups	56
		 Acetylation Trifluoroacetates Trimethylsilyl ether and related derivatives Isopropylidene compounds n-Butylboronate derivatives 	56 57 57 58 58

		Contents	ix
	D.	Derivatives of Fatty Aldehydes	58
		 Hydrazone derivatives Acetals 	58 59
	E.	Derivatives of Double Bonds	59
*		 Mercuric acetate derivatives Hydroxylation Epoxidation Hydrogenation Deuterohydrazine reduction 	59 60 60 61 61
 5.	The .	Analysis of Fatty Acids	63
	<i>A</i> .	Introduction	63
	В.	Analytical Gas-Liquid Chromatography	63
		 The common fatty acids of plant and animal origin Some less common fatty acids Spurious peaks on recorder traces 	63 70 72
	C.	Isolation of Individual Fatty Acids for Structural Analysis 1. Adsorption chromatography	73. 73
		 Silver nitrate chromatography Preparative gas chromatography Liquid-liquid partition chromatography (including HPLC and TLC) 	74 76 77
	D.	Spectroscopy of Fatty Acids	79
		 Infrared absorption and Raman spectroscopy Ultraviolet spectroscopy Nuclear magnetic resonance spectroscopy Mass spectrometry 	79 80 81 82
	E.	Identification of Fatty Acids by Chemical Degradative Procedures	84
	·	 Chain-length determination Location of double bonds in fatty acid chains Location of other functional groups in fatty acids 	84 84 87
	F.	Physical characterization of Fatty acids	89
	G.	Preparation of Large Quantities of Pure Fatty Acids	89
		 Saturated fatty acids Polyunsaturated fatty acids 	89 90
6.	The	Analysis of Simple Lipid Classes	93
	A.	Chromatographic Separation of the Common Simple Lipid Classes	93
		 Thin-layer chromatography Column chromatography High-temperature gas-liquid chromatography 	93 96 98

X Contents

	B.	The Analysis of Individual Simple Lipids and of their Hydrolysis Products	99
		 Unesterified (free) fatty acids Boundfatty acids Glycerides and glycerol Cholesterol 	99 99 99 100
'au	<i>C</i> .	Some Special Cases	101
		 Preparative separation of isomeric mono- and di-acylglycerols Alkyldiacylglycerols and neutral plasmalogens Wax esters and related compounds 	101 101 105
*			107
7.	The	Analysis of Complex Lipids	107
	A.	Preliminary Separation and Preparation of Lipid Samples	107
		 Simple group separations Cations associated with complex lipids 	107 108
	В.	Group Separation by Column Chromatography	109
-		 Preparative chromatography on silicic acid columns Preparative chromatography on Florisil and alumina columns DEAE, TEAE and CM-cellulose chromatography HPLC of complex lipidr 	109 110 111 114
	C.	Thin-Layer Chromatography of Complex Lipidr	115
		 Single-dimensionalTLC systems Two-dimensional TLC systems Location and identification of complex lipidr on TLC plates 	115 117 119
	D.	Determination of Complex Lipids Separated by Chromatographic Procedures	121
		 Universal procedures Phospholipid determination by phosphorus assay Glycerophospholipid determination by glycerol assay Nitrogenous bases of phospholipids Determination of glycosphingolipidr and sphingomyelin 	121 121 122 123 123
	E.	The Analysis of Glycosphingolipids, Sphingomyelin and their Hydrolysis Products	123
		 Cerebrosides and oligoglycosylceramides Gangliosides Long-chain bases The fatty acid components of glycolipids The analysis of hexoses 	123 126 127 129 130
	F.	Some Specific Problems in the Analysis of Phospholipids	131
		 Alkyl- and alkenyl-ether forms of phospholipids Analysis of glycerophosphorylmoieties Phosphonolipids Polyphosphoinositides 	131 133 133 134

		Contents	хi
8.	The Analysis of Molecular Species of Lipids		135
	Α.	Introduction	135
	В.	General Methods of Analysis	136
		 Liquid-solid and liquid-liquid chromatography Gas-liquid chromatography of intact lipids 	136 136
	C.	Molecular Species of Simple Lipids	139
		 Cholesterol esters and wax esters Monoacylglycerols Diacylglycerols Triacylglycerols 	139 140 140 143
	D.	Molecular Species of Complex Lipids	148
,	1986 Turk	 General approaches to the problem Phosphatidylcholine Phosphatidylethanolamine Other glycerophosphatides Glycosyldiacylglycerols Sphingomyelin and glycosphingolipids 	148 149 150 150 151
9.	Stri	actural Analysis of Lipids by means of Enzymatic Hydrolysis	155
0.	<i>A</i> .	Introduction	155
	В.	Positional Distribution of Fatty Acids in Triacylglycerols	156
		 Pancreatic lipase hydrolysis Lipase of Rhizopus arrhizus Stereospecific analysis of triacylglycerols 	156 157 158
	<i>C</i> .	Enzymatic hydrolysis of complex lipids	161
		 Phospholipase A, Pancreatic lipase and related enzymes Phospholipase C Phospholipase D 	161 163 164 165
	D.	The use of Enzymatic Hydrolysis in the Determination of Molecular Species of Lipids	166
10.	The	Analysis and Radioassay of Isotopically-labelled Lipids	167
	A.	Introduction	167
	В.	Chromatography and Radioassay	168
		 Thin-layer chromatography Gas-liquid chromatography Liquid column chromatography 	168 170 173
	C.	Location of ¹⁴ C in Aliphatic Chains	173

xii

Contents

11.	The Separation of Plasma Lipoproteins		177
	A.	The Nature of Plasma Lipoproteins	177
	В.	Separation by Ultracentrifugation	178
	C.	Separation by Electrophoresis	178
34 	D.	Precipitation of Lipoproteins by Polyanions	179
	E.	Analysis and Quantification of Lipoproteins	179
App	endix .	A: Commercial Sources of Lipids	181
Appe	endix i	B: Sources of Information	183
References			185
INDEX			199