Contents

The Use of Combined Gas Chromatography-Mass Spectrometry in the Analysis of Plant Growth Substances

P. HEDDEN (With 8 Figures)

1	Introduction
2	Identification by GC-MS
	2.1 Derivatisation
	2.1.1 Methylation
	2.1.2 Trimethylsilylation
	2.1.3 Permethylation
	2.2 Gas Chromatography
	2.3 Qualitative Mass Spectrometry
	2.4 Quantitative Mass Spectrometry
3	Gibberellins
	3.1 Extraction and Purification
	3.2 GC-MS
4	Abscisic Acid and Related Compounds
	4.1 Qualitative GC-MS
	4.2 Quantitative GC-MS
5	Brassinosteroids
R	eferences

Applications of Mass Spectrometry for the Examination of Pectic Polysaccharides

R. R. SELVENDRAN and B. J. H. STEVENS (With 6 Figures)

1	Introduction	23
2	Structural Analysis of Pectic Polysaccharides	23
3	Determination of the Nature of the Glycosidic Linkages	24
4	Separation and Identification of Partially Methylated Alditol Acetates .	27
5	Extensions of Methylation Analysis	29
	5.1 Controlled Partial Acid Hydrolysis Studies	29
	5.2 β -Eliminative Degradation Studies	
	5.2.1 Neutral Glycosyl Residues Linked to Galacturonosyl Residues	32
	5.2.2 Glycosyl Residues Linked to 0-4 of 2,4-Linked	
	Rhamnopyranosyl Residues	32
6	Sequencing of Sugar Residues in Pectins	33
	6.1 Partial Acid Hydrolysis	33
	6.1.1 Characterisation of Oligosaccharides as Permethylated	
	Derivatives	34

7 5 7 5 8 1	5.2 Partial Acetolysis 5.3 Enzymatic Hydrolysis 5.3 Enzymatic Hydrolysis 5.3 Enzymatic Hydrolysis Sequencing of Pectic Polysaccharides by Partial Depolymerisation of Permethylated Derivatives Experimental ferences	37 38 38 40 43
and	C-MS Methods for Cyclic Nucleotides in Higher Plants I for Free High Unsaturated Fatty Acids in Oils JANISTYN (With 10 Figures)	
2	Introduction	47 48
	(Zea mays)	48 51
5	Stability of Cyclic Purine Nucleotides in the Presence of Hydrochloric Acid During Extraction	55
	Guanosine-3': 5'-Monophosphate (cGMP) in Maize Seedlings (Zea mays)	56
	GC-Separation of Synthetic cAMP and cGMP in a Mixture	59
	Cyclic Pyrimidine Nucleotides in Plants?	60
	Free High Unsaturated Fatty Acids in Oils	
10	Conclusions	64

GC-MS Methods for Lower Plant Glycolipid Fatty Acids

H. NYBERG (With 4 Figures)

1	Introduction					67
2	Extraction of the Plant Material					67
	2.1 Handling and Storage					67
	2.2 Extraction with Organic Solvents	÷			•	68
	2.3 Purification of the Extract					69
3	Separation of Glycolipids from the Total Lipid Extract.					70
	3.1 Column Chromatography			•		70
	3.2 Thin-Layer Chromatography (TLC)					72
	3.3 Other Applications					73
4	Isolation of the Glycolipids					74
	4.1 Thin-Layer Chromatography					74
	4.2 Localization of the Glycolipids on TLC.					75
	4.3 Removing the Spots from the TLC Plates					76
5	Derivatization of the Glycolipid Fatty Acids for GC-MS					76
	5.1 General Features					76
	5.2 Formation of Methyl Esters					77
	5.3 Silylation and Other Methods					77

. 65

6	GLC Instrumentation for Fatty Acid Analysis	
	6.1 General Features	
	6.2 Carrier Gas System	. 79
	6.3 Injection and Injectors	. 79
	6.4 The Detector	. 82
7	Column Selection for Fatty Acid GLC Analysis	. 83
	7.1 Column Types	. 83
	7.2 Supports, Liquid Phases, and Their Characteristics	. 84
8	Interpretation of GC Data and Calculation of Results	. 87
	8.1 Identification of Peaks Using Standard Compounds	
	8.2 Quantitation of Results	
	8.3 External and Internal Standardization	. 89
9	Fatty Acid Ester Structure Determination by GC-MS	
-	9.1 Equivalent Chain Lengths (ECL)	
	9.2 Semilogarithmic Correlations	
	9.3 Mass Spectrometers and Their Function Principles	
	9.4 Interpretation of Mass Spectra of Fatty Acid Esters	
10	Abbreviations.	
	eferences	
***		. ,,
an	nalysis of Phospholipid Molecular Species by Gas Chromatography ad Coupled Gas Chromatography-Mass Spectrometry V. V. LYNCH and G. A. THOMPSON, Jr. (With 6 Figures)	
1	Introduction	. 100
	Lipid Preparation	
	2.1 Lipid Extraction	. 101
	2.2 Purification of Phospholipids	. 102
	2.2.1 Column Chromatography	
	2.2.2 Thin Layer Chromatography	. 103
3	Formation of Derivatives for GC or GC-MS	. 104
	3.1 Phospholipase C Treatment	
	3.2 Conversion of Diacylglycerols to Silyl Derivatives	. 106
	3.2.1 Formation of Trimethylsilyl Derivatives	. 107
	3.2.2 Formation of tert-Butyldimethylsilyl Derivatives	. 107
4	Gas Chromatography	. 108
5	Mass Spectrometry	. 110
	5.1 Instrumentation	. 111
	5.2 Operating Conditions	. 111
	5.3 Identification of Molecular Species	. 111
	5.4 Quantitation of Molecular Species by GC-MS	
	5.5 Quantitation of Molecular Species by GC-MS Following Reduction	ı
	of Double Bonds Using Deuterium	. 115
	5.6 Direct MS Analysis of Underivatized Phospholipids	
6	Determination of Positional Distribution of Acyl Chains	
	Using Phospholipase A ₂	. 117

.

GC-MS of Plant Sterol Analysis

G. COMBAUT (With 2 Figures)

1	Introduction
2	Development of GC-MS Plant Sterol Analysis
3	Operations Before GC-MS Sterol Analysis
	3.1 Extraction and Isolation of Plant Sterols
	3.2 Free Sterols and (or) Sterols from Steryl-Esters
	3.3 Purification of Sterolic Fractions
	3.4 Derivatization
4	Characterization of Sterols
	4.1 Characterization of Sterols by GC Data
	4.2 Characterization of Sterols by MS Data
	4.3 Characterization of Sterols by GC and MS Data
	4.3.1 A Typical Analysis of 4-Demethyl and 4,4-Dimethyl Sterols
	from Zea mays
	4.3.2 Co-Occurrence of Δ^5 - and Δ^7 -Sterols in Tracheophytes 128
	4.3.3 Side Chain-Hydroxylated Sterols from Red Algae 129
	4.3.4 4-Methyl Sterols of Dinoflagellates
5	Conclusion
R	eferences

GC-MS Methods for Terpenoids

L. WITTE

1	Introduction
2	Isolation Methods
3	Prefractionation and Ancillary Reactions
4	Gas Chromatography
5	Retention Data
6	Mass Spectrometry
R	eferences

GC-MS of Auxins

L. RIVIER (With 22 Figures)

1	Introduction
2	The Compounds Involved
3	Reference Compounds
4	Extraction
5	Purification
6	Columns for GC
7	Injection Techniques
8	Derivatisation
9	Interface Between GC and MS
10	Mass Spectrometer
	Data Systems
12	Ionization

13	GC-MS Strategy for Au	xin	Α	na	lys	sis														165
14	Quantification															•				176
15	The Internal Standard .																	•		178
16	Experimental Procedure	•																		182
17	Conclusions	•			•			•	•					•			•			185
Ref	erences	•	•	•	•	•	•	•		•	•	٠	•	•	•	•			•	185

GC-MS Methods for the Quantitative Determination and Structural Characterization of Esters of Indole-3-Acetic Acid and myo-Inositol R. S. BANDURSKI and A. EHMANN (With 4 Figures)

1	Introduction	•	189
	1.1 Discovery of IAA-Inositols	. 1	189
	1.2 Occurrence of IAA Conjugates	. 1	190
	1.3 Importance of Measuring and Identifying Hormone Conjugates .	•	191
2	Quantitative Analysis and Identification of the IAA-Inositols		191
	2.1 Analysis After Hydrolysis		191
	2.1.1 Methods for Hydrolysis of IAA Conjugates	•	192
	2.1.2 Use of Internal Standards	. 1	194
	2.2 Analysis Before Hydrolysis		195
	2.2.1 A Quantitative Estimation of IAA-Inositol Using [³ H]-IAA-		
	myo-Inositol as an Internal Standard	. 1	195
3	Qualitative Analysis of IAA-Inositols	•	195
	3.1 The Inositol Moiety	•	195
	3.2 Derivitization of IAA-Inositols for GC-MS	•	196
	3.3 Mass Spectral Fragmentation Pattern		
	3.3.1 I-DL-1-O-(Indole-3-Acetyl)-myo-Inositol (6 TMS, MW 769)	. 2	201
	3.3.2 2-O-(Indole-3-Acetyl)-myo-Inositol (6 TMS MW 769)	. 2	201
	3.3.3 Di-O-[N-(Trimethylsilyl) Indole-3-Acetyl]-O-Tetra-O-		
	Trimethylsilyl-myo-Inositol	. 2	201
	3.3.4 Tri-O-[N-(Trimethylsilyl) Indole-3-Acetyl]-O-Tri-O-		
	Trimethylsilyl-myo-Inositol	. 2	206
	3.3.5 IAA-myo-Inositol-Arabinoside and IAA-myo-Inositol-		
	Galactoside		
	3.4 Uses of GC-MS to Identify and Characterize IAA-Esters	. 2	206
4	Conclusions		210
5	Abbreviations	. 2	211
R	leferences		211

GC-MS Methods for Cytokinins and Metabolites

L. M. S. PALNI, S. A. B. TAY, at	d J. K. MACLEOD (With 8 Figures)
----------------------------------	----------------------------------

1	Introduction	4
2	Gas Chromatography (GC)	0
	2.1 Instrumentation	
	2.1.1 Liquid Stationary Phases and Columns	1
	2.1.2 Injectors	2
	2.1.3 Detectors \ldots \ldots \ldots \ldots \ldots \ldots \ldots 22^{4}	4

2.2 Derivatisation of Cytokinins	. 225
2.2.1 Trimethylsilyl (TMSi) Derivatives	. 225
2.2.2 Permethyl Derivatives	. 227
2.2.3 tertButyldimethylsilyl (t-BuDMSi) Derivatives	228
2.2.4 Trifluoroacetyl (TFA) Derivatives	
2.3 Preparative GC.	
3 Mass Spectrometry	
3.1 Instrumentation	
3.1.1 Sample Introduction	
3.1.2 Ionisation Methods	
3.1.3 Analysers	
3.1.4 Data Systems	
3.2 Combined Gas Chromatography-Mass Spectrometry (GC-MS) .	
4 Applications of Mass Spectrometry in Cytokinin Analysis	
4.1 Structural Studies	
4.2 Quantification of Cytokinins	. 236
4.2.1 Internal Standards	. 237
4.2.2 Stable Isotope Dilution Mass Spectrometry	
4.2.3 Quantification Using GC-MS	
4.2.4 Probe Analysis	
4.3 Metabolic Profiling	
5 General Remarks and Conclusion	
References.	
	. 475

GC-MS Method for Volatile Flavor Components of Foods

H. KAMEOKA (With 8 Figures)

1	Introduction	4
2	GC-MS Methods	4
	2.1 Preparation Methods of Flavor Samples	5
	2.2 Operational Methods	6
3	Volatile Flavor Components	6
	3.1 Fruits	6
	3.2 Vegetables	3
	3.3 Mushrooms	8
	3.4 Tea	0
	3.5 Beans and Nuts	1
	3.6 Grains	1
	3.7 Jams	2
	3.8 Fermentation Products	3
R	eferences	4

GC-MS Methods for Tobacco Constituents

H. KODAMA (With 20 Figures)

1	Introduction														277
2	Cembranoids and	Their	De	egra	ded	l Co	mp	our	nds	•	•			•	277

3 Labdanoids and The																							
4 Carotenoid-Degraded																							
5 Sesquiterpenoids																							
6 Terpenoid Glycoside																							
7 Linked Scanning																							
References	•	•	•	•	·	·	·	·	٠	·	•	٠	·	•	•	·	·	•	•	٠	·	٠	298
Caller A Taller																							200
Subject Index	·	·	·	·	·	·	·	٠	٠	·	·	٠	·	·	·	·	·	·	·	·	·	·	299