

2684-8039

High Performance Liquid Chromatography in Plant Sciences

Edited by
H.F. Linskens and J.F. Jackson

Contributors

G.W.M. Barendse Ch.-M. Chen R.A. Creelman
A. Crozier P.J. Davies A. Ernstsén A.W. Galston
R.D. Hartley J.P.F.G. Helsper L.E. Hood S.B.H. Kent
J.M. Robertson G. Sandberg L.A. Smith M.A. Smith
R.N. Strange R. Sütfeld B. Sundberg P. Tempst
G.A. Thompson, Jr. A.F. Tiburcio J.A.D. Zeevaart

With 74 Figures



Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo

Contents

High Performance Liquid Chromatography of Gibberellins

G. W. M. BARENDSE (With 8 Figures)

1	Introduction	1
2	Extraction and Purification	5
3	Group Separation Procedures	7
3.1	PVP Adsorption Chromatography	7
3.2	Gel Permeation or Steric Exclusion Chromatography	7
3.3	Anion Exchange Chromatography	8
3.4	Charcoal Adsorption Chromatography	8
3.5	Sephadex G-10 Chromatography	8
3.6	Countercurrent Distribution	9
3.7	Silica Gel Coated Disposable Extraction Columns	9
4	High Performance Liquid Chromatography (HPLC)	9
4.1	Gel Permeation or Steric Exclusion HPLC	10
4.2	Normal Phase HPLC	11
4.3	Reversed Phase HPLC	12
4.4	Detection of Gibberellins After HPLC	14
5	Recent Developments and Prospects	19
5.1	Diode Array UV Detection	20
5.2	Electrochemical Detection	20
5.3	Combined HPLC Immunoassay	20
	References	21

Characterization of Cytokinins and Related Compounds by HPLC

CH.-M. CHEN (With 8 Figures)

1	Introduction	23
1.1	Background	23
1.2	Structure and Physicochemical Properties of Cytokinins and Related Compounds	23
2	Cytokinin Isolation and Sample Preparation	25
2.1	Cytokinin Isolation	25
2.2	Sample Preparation	27
3	Preparation of Mobile Phase	27
3.1	Water	27
3.2	Organic Solvents	27
4	Chromatography	28
4.1	Column Selection	28
4.2	Separation Examples	28

4.2.1 Ion Exchange HPLC	28
4.2.2 Normal Phase HPLC	31
4.2.3 Reverse Phase HPLC	33
4.2.4 Ion-Pair Reverse Phase HPLC	35
5 Concluding Remarks	37
References	37

Separation and Purification of Abscisic Acid and Its Catabolites by High Performance Liquid Chromatography

R. A. CREELMAN and J. A. D. ZEEVAART (With 5 Figures)

1 Introduction	39
2 Abscisic Acid and Its Catabolites	41
3 Use of High Performance Liquid Chromatography to Characterize Abscisic Acid and Its Catabolites	44
3.1 Synthesis of (\pm)-Abscisic Acid and Catabolites	44
3.2 Resolution of (\pm)-Abscisic Acid	44
3.3 Methods for the Purification of Abscisic Acid and Catabolites by High Performance Liquid Chromatography	45
4 Concluding Remarks	48
References	49

The Determination of Abscisic Acid by High Performance Liquid Chromatography

J. M. ROBERTSON

1 Introduction	52
1.1 HPLC and ABA	52
1.1.1 Why Have Many Different Methods Been Used?	52
1.1.2 Which Method is Correct? How to Choose	52
1.1.3 General Approach to HPLC of ABA	53
1.2 HPLC General	53
1.2.1 Theoretical Considerations	53
1.2.1.1 Retention	53
1.2.1.2 Band Spreading	54
1.2.1.3 Resolution	54
1.2.1.4 Controlling R_s by N , k' , and α	55
1.2.2 Preparative Versus Analytical Operation	56
1.2.3 Elution	56
1.2.4 The Different Modes of HPLC	58
1.2.4.1 Partition or Liquid-Liquid Chromatography (LLC)	58
1.2.4.2 Adsorption or Liquid-Solid Chromatography (LSC)	58
1.2.4.3 Size Exclusion Chromatography (SEC)	59
1.2.4.4 Ion Exchange Chromatography (IEC)	59
1.2.4.5 Bonded-Phase Chromatography (BPC)	60
1.2.5 Detection	60
1.3 ABA General	61
1.3.1 The ABA Molecule	61

1.3.2	Extraction and Preparation	62
1.3.3	Determination of ABA	62
1.4	Generalized Scheme for the Separation of ABA	63
1.4.1	Choosing the Columns	64
1.4.2	Typing the System	64
1.4.3	Extraction and Preparation for RPLC	65
1.4.4	The Second Column	67
1.4.5	Chromatography of ABA in Conjunction with Other Compounds	68
1.4.6	Summary of General Separation Scheme	68
	References	68

High Performance Liquid Chromatography and the Analysis of Indole-3-Acetic Acid, and Some of Its Decarboxylated Catabolites in Scots Pine (*Pinus sylvestris* L.)

G. SANDBERG, A. CROZIER, A. ERNSTSEN, and B. SUNDBERG
(With 11 Figures)

1	Introduction	72
2	Internal Standardization	74
3	Extraction and Purification	76
4	High Performance Liquid Chromatography	77
5	Examples	79
5.1	Indole-3-Acetic Acid	79
5.1.1	Extraction	79
5.1.2	Purification	79
5.1.2.1	PVP, XAD-7 and Sep-Pak	80
5.1.2.2	Immunoaffinity Chromatography	80
5.1.3	HPLC Analysis	81
5.2	Indole-3-Carboxylic Acid	83
5.2.1	Extraction and Purification	83
5.2.2	HPLC Analysis	84
5.2.3	Internal Standards	85
5.3	Indole-3-Methanol	86
5.3.1	Extraction and Purification	86
5.3.2	HPLC Analysis	87
5.3.3	Internal Standards	88
6	Conclusions	89
	References	89

HPLC for the Separation and Determination of Phenolic Compounds in Plant Cell Walls

R. D. HARTLEY (With 2 Figures)

1	Introduction	92
2	General Considerations	94
3	The Recommended Procedure – Isolation of Cell Walls	95
3.1	Reagents and Equipment	95

3.1.1 Reagents (All Analytical Grade)	95
3.1.2 Equipment	95
3.2 Isolation of Cell Walls	96
3.3 Isolation of Dietary Fibre	96
4 The Recommended Procedure – Release of Phenolic Acids and Aldehydes from Cell Walls and Their Separation and Determination by HPLC (C ₁₈ Reverse Phase) (<i>Methods 1 and 2</i>)	97
4.1 Reagents and Equipment	97
4.1.1 Reagents (All Analytical Grade)	97
4.1.2 Equipment	97
4.2 Release of Phenolic Acids and Aldehydes from Cell Walls and Preparation of Solutions for Analytical HPLC (C ₁₈ Reverse Phase)	97
4.3 Analytical HPLC (C ₁₈ Reverse Phase) of Phenolic Acids and Aldehydes (<i>Methods 1 and 2</i>)	97
5 Alternative Procedure – Release of Phenolic Acids and Aldehydes from Cell Walls and Their Separation and Determination by HPLC (PRP-1 Resin) (<i>Methods 3 and 4</i>)	98
5.1 Reagents and Equipment	98
5.2 Release of Phenolic Acids and Aldehydes from Cell Walls and Preparation of Solutions for HPLC (PRP-1 Resin)	98
5.2.1 Method for Analytical HPLC (PRP-1 Resin)	98
5.2.2 Method for Preparative HPLC (PRP-1 Resin)	99
5.3 Analytical HPLC (PRP-1 Resin) of the Phenolic Acids and Aldehydes	99
5.3.1 Phenolic Aldehydes and trans,trans-Diferulic Acid (<i>Method 3</i>)	99
5.3.2 p-Coumaric and Ferulic Acids (<i>Method 4</i>)	99
5.4 Preparative HPLC (PRP-1 Resin) of the Phenolic Acids and Aldehydes	101
5.4.1 Preparation of the Aldehydes and trans,trans-Diferulic Acid	101
5.4.2 Preparation of p-Coumaric and Ferulic Acids	101
6 Amount of Phenolic Acids and Aldehydes Released from the Cell Walls of Graminaceous Plants	101
References	101

HPLC of Thiophenes for Phytochemical and Biochemical Research

R. SÜTFELD (With 6 Figures)

1 Introduction	104
2 Material and Methods	105
3 Use of HPLC for Phytochemical Research on Thiophenes	106
3.1 Preliminary Experiments: HPLC of Commercially Available Reference Substances	106
3.2 HPLC of Naturally Occurring Thiophenes	107
3.2.1 Thiophenes from <i>Tagetes patula</i> Seedlings	107
3.2.2 Thiophenes from Other Plant Sources	109
4 Use of HPLC for Enzymatic Research on Thiophenes	111
5 Conclusions	112
References	112

High Performance Liquid Chromatography of Ascorbic Acid

J. P. F. G. HELSPER

1	Introduction	114
2	Sample Preparation	114
3	Stationary and Mobile Phases	115
3.1	Separation of Ascorbic Acid from Dehydroascorbic Acid and from Reducing Agents	115
3.2	Separation of Ascorbic Acid and Metabolically Related Compounds	116
3.3	Separation of Isomeric Forms of Ascorbic Acid	117
4	Detection Techniques for Ascorbic Acid	117
	References	119

High Performance Liquid Chromatography of Phytoalexins

R. N. STRANGE (With 13 Figures)

1	Introduction	121
2	Instrumentation	122
3	Preparative HPLC of Phytoalexins	125
3.1	Principles	125
3.1.1	Elicitation	125
3.1.2	Extraction and Clean Up	126
3.1.3	Chromatography	127
3.2	Practice	128
3.2.1	Isolation of Stilbene Phytoalexins from Kernels	128
3.2.2	Isolation of Hydroxyflavan Phytoalexins from Narcissus Bulbs	129
3.2.3	Isolation of Pterocarpin Phytoalexins (Glyceollins) from Soybean Cotyledons	130
3.2.4	Isolation of Isoflavone and Isoflavanone Phytoalexins from Pigeonpea Seeds	132
3.2.5	Isolation of Chalcone and Stilbene Phytoalexins from Pigeonpea Leaves	133
4	Analytical HPLC of Phytoalexins	134
4.1	Principles	134
4.1.1	Elicitation	134
4.1.2	Sampling	134
4.1.3	Extraction and Clean Up	135
4.1.4	Chromatography	136
4.2	Practice	137
4.2.1	Analytical HPLC of Furanoacetylenic Phytoalexins from Broad Bean	137
4.2.2	Analytical HPLC of Pterocarpin Phytoalexins (Glyceollins) from Soybean	139
4.2.3	Analytical HPLC of Phytoalexins from Potato	141
4.2.4	Analytical HPLC of Phytoalexins from French Bean	142
4.2.5	Analytical HPLC of Phytoalexins from Cotton	142

5 Application of HPLC to the Solution of Outstanding Problems in Phytoalexin Research	144
5.1 Assessment of the Role of Phytoalexins in Resistance to Microbial Attack	144
5.2 Measurement of the Phytoalexin Potential of the Plant	145
5.3 The Elicitation, Biosynthesis and Degradation of Phytoalexins	145
6 Concluding Remarks	146
References	146

Analysis of Lipids by High Performance Liquid Chromatography

L. A. SMITH and G. A. THOMPSON, JR. (With 4 Figures)

1 Introduction	149
2 Instrumentation	150
2.1 Pumps, Injectors, and Columns	150
2.2 Detectors	150
2.3 Mobile Phase Selection	154
3 Lipid HPLC Applications	156
3.1 Initial Lipid Extraction and Purification	156
3.1.1 Lipid Extraction	156
3.1.1.1 Comments	156
3.1.1.2 Procedure	156
3.2 Separation of Lipid Classes	157
3.2.1 HPLC Separation of Lipid Classes	157
3.2.2 Separation of Lipid Classes by Other Techniques	158
3.2.2.1 Column Chromatography	158
3.2.2.2 Thin Layer Chromatography	159
3.3 HPLC Analysis of Individual Molecular Species of Lipid Classes	160
3.3.1 Separation of Phospholipid Molecular Species by HPLC of Diglyceride Derivatives	160
3.3.1.1 Procedure	161
3.3.1.2 Comments	161
3.3.2 HPLC Separation of Phosphatidylglycerol Molecular Species	161
3.3.2.1 Sample Preparation	161
3.3.2.2 Instrumentation and Mobile Phases	162
3.3.3 HPLC Separation of Glycolipid Molecular Species	163
3.3.3.1 Sample Preparation	163
3.3.3.2 Instrumentation and Mobile Phase	163
3.3.4 Fatty Acid Analysis	164
3.3.4.1 Production of Free Fatty Acids from Lipid Classes	164
3.3.4.2 Preparation of Fatty Acid Derivatives	164
3.3.4.3 HPLC Analysis of Nitrophenacyl Fatty Acid Derivatives	164
3.3.4.4 Trace Analysis of Fatty Acid by HPLC	166
3.3.5 HPLC of Other Lipid Classes	166
References	167

Practical High Performance Liquid Chromatography of Proteins and Peptides

P. TEMPST, L. E. HOOD, and S. B. H. KENT (With 7 Figures)

1	Introduction	170
2	Size Exclusion HPLC	171
2.1	Columns	172
2.1.1	Packings and Surface Interactions	172
2.1.2	Fractionation Limits	172
2.2	Operational Parameters and Resolution	174
2.2.1	Mobile Phase	174
2.2.2	Sample	176
2.2.3	Mobile Phase Velocity	176
2.3	Practical Applications	176
2.3.1	Molecular Weight Determination	176
2.3.2	Preservation of Biological Activity	177
2.3.3	Selected Applications	177
2.4	Large-Scale Separations	178
2.5	Maintenance	179
3	Ion Exchange HPLC	179
3.1	Columns	180
3.2	Operational Parameters and Resolution	181
3.2.1	Mobile Phase	181
3.2.2	Mobile Phase Velocity	183
3.2.3	Gradient Slope	183
3.2.4	Sample and Load	183
3.3	Selected Applications	184
4	Reversed Phase HPLC	185
4.1	Columns	186
4.2	Operational Parameters and Resolution	192
4.2.1	Mobile Phase	192
4.2.1.1	Organic Solvent	192
4.2.1.2	Mobile Phase pH	193
4.2.1.3	Ion-Pairing Agents	194
4.2.1.4	Other Aqueous Solvent Compositions	195
4.2.1.5	Acid Concentration	195
4.2.2	Mobile Phase Velocity and Temperature	196
4.2.3	Gradient Slope	196
4.2.4	Sample Composition and Load	197
4.2.5	Prediction of Retention Times	198
4.3	High Sensitivity RP-HPLC	198
4.4	Applications	199
4.4.1	Two-Dimensional RP-HPLC	199
4.4.2	Hydrophobic Polypeptides	200
4.4.3	Others	201
4.5	Maintenance	202
5	Multidimensional HPLC	202
	References	204

Monitoring Polyamines in Plant Tissues by High Performance Liquid Chromatography

M. A. SMITH and P. J. DAVIES (With 7 Figures)

1	Introduction	209
2	High Performance Liquid Chromatography of Dansylamines	210
2.1	Dansyl Derivatives	210
2.2	Reagents and Stock Solutions	211
2.3	Extraction of Polyamines	212
2.4	Purification of Extracts	212
2.5	Dansylation of Amines in a Sample	213
2.6	Chromatographic Separation of Dansylamines	214
2.7	Quantification	216
3	High Performance Liquid Chromatography of Benzoylamines	217
3.1	Benzoylation of Amines in a Sample	218
3.2	Chromatographic Separation of Benzoylamines	218
4	Ion-Pair Reverse Phase Chromatography of Underivatized Polyamines	219
4.1	o-Phthalaldehyde Derivatives	220
4.2	Sample Preparation	220
4.3	Chromatographic Separation of Underivatized Polyamines	221
4.4	Post-Column Derivatization with OPA	221
4.5	Detection of OPA Derivatives	221
5	Ion-Exchange Chromatography of Underivatized Polyamines	222
5.1	Chromatographic Separation of Underivatized Amines and Related Compounds	222
6	Conclusion	223
	References	224

Analysis of Alkaloids in Tobacco Callus by HPLC

A. F. TIBURCIO and A. W. GALSTON (With 3 Figures)

1	Introduction	228
1.1	Background	228
1.2	Analytical Methods	229
2	Experimental	230
2.1	Plant Material	230
2.1.1	Explant Origin and Callus Induction	230
2.1.2	Callus Culture	230
2.2	Alkaloid Extraction	231
2.2.1	In Dry Tissue	231
2.2.2	In Fresh Tissue	231
2.3	Analysis by HPLC	231
2.3.1	Sample Preparation and Purification of Standards	231
2.3.2	Instrumentation and Separation of Alkaloids	232
2.3.3	Alkaloid Quantitation. Calibration Curves	232
3	Results and Discussion	232
3.1	Callus Culture Method	232
3.2	Alkaloid Extraction	233

3.3 Analysis by HPLC	234
3.4 Applications to the Study of Alkaloid Production and Metabolism In Vitro	236
3.4.1 In Vitro Biosynthesis of Nornicotine	236
3.4.2 Effect of Organic Acids on Alkaloid Content	237
3.4.3 Effect of Putrescine Biosynthetic Inhibitors on Alkaloid Content	238
4 Conclusions	239
References	240
Subject Index	243