



*JOURNAL OF CHROMATOGRAPHY
LIBRARY - volume 57*

*retention and selectivity
in liquid chromatography*

*prediction, standardisation and
phase comparisons*

*edited by
Roger M. Smith*

ELSEVIER

JOURNAL OF CHROMATOGRAPHY LIBRARY—volume 57

retention and selectivity in liquid chromatography

prediction, standardisation and phase comparisons

edited by

Roger M. Smith

*Department of Chemistry, Loughborough University of Technology, Loughborough,
Leicestershire LE11 3TU, UK*



ELSEVIER

Amsterdam — Lausanne — New York — Oxford — Shannon — Tokyo 1995

ELSEVIER SCIENCE B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

ISBN 0-444-81539-2

© 1995 Elsevier Science B.V. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the publisher, Elsevier Science B.V., Copyright & Permissions Department, P.O. Box 521, 1000 AM Amsterdam, The Netherlands.

Special regulations for readers in the U.S.A. – This publication has been registered with the Copyright Clearance Center Inc. (CCC), Salem, Massachusetts. Information can be obtained from the CCC about conditions under which photocopies of parts of this publication may be made in the U.S.A. All other copyright questions, including photocopying outside of the U.S.A., should be referred to the Publisher.

No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein.

This book is printed on acid-free paper.

Transferred to digital printing 2006

JOURNAL OF CHROMATOGRAPHY LIBRARY — volume 57

retention and selectivity in liquid chromatography

prediction, standardisation and phase comparisons

Preface

This book grew out of a long standing interest in the ways in which retention and the selectivity of separation in liquid chromatography are dependent on the structure of the analyte and on changes in the mobile and stationary phases. These relationships are at the heart of an understanding of the operation of liquid chromatography and of the ways in which the chromatographer can manipulate the conditions of a separation to achieve the analysis of a complex sample.

The factors involved in these processes are complex and even 90 years after the pioneering work of Tswett are still not fully understood. Any progress is linked to the development of an understanding of the physical chemical process of solvation and the physicochemical nature of the stationary and mobile phases. Chromatography is also a valuable practical analytical method and much can be learnt by studying relative interactions and by comparing the behaviour of analytes with different chemical structures under different separation conditions. To achieve this objective, techniques for recording relative retentions are needed so that results can be reproduced in different laboratories or by different operators.

However, liquid chromatography has a notoriously poor transferability, the same high versatility which enables separations to be precisely optimized also means that small changes between systems can alter the separations. This book addresses some of the ways in which these problems have been overcome to enable retention predictions, identifications and the characterization of the properties of mobile and stationary phases, to be carried out. The work owes much to studies in gas chromatography, in particular the work of Kováts in providing a retention index scale and of Rohrschneider and McReynolds on the comparison of stationary phases.

A theme which leads through the different chapters is the value of relative measurements. Most obviously in the descriptions of the different retention index scales in liquid chromatography and their application to the identification of a wide range of analytes. The indices also form the basis of one of the studies on retention prediction, the other relating retention to the contribution of analytes to partition coefficients. Related methods have been used to compare analytes and their interaction properties. The final group of chapters investigates methods for the comparison of mobile and stationary phases not just by using a simple solvent strength parameter but by examining the comparative interaction of the phases to different types of analytes either in terms of their shapes or physical properties.

Bringing these chapters together enables the different approaches to be compared and illustrates the values of each. Hopefully, this will stimulate further research or different approaches for this is by no means the full description of the mechanism of retention. Much more still needs to be done, in particular to understand how complex molecules behave. In this case, the chromatographic behaviour of the analyte under different conditions may itself provide valuable information about the physical properties of the analyte.

I would like to thank many of the contributors for useful and interesting discussion of their work and the stimulation it has provided for our own studies. I would also thank my research and project students at Loughborough University of Technology, who have contributed to our own studies in this field. In the same way that their individual contributions have together built our overall study, so I hope that the chapters of this book will contribute to an overall greater understanding of the retention process in liquid chromatography.

Roger M. Smith
May 1994

List of Contributors

- M. BOGUSZ *Institut für Rechtsmedizin, Medizinische Fakultät, Rheinisch-Westfälische Technische Hochschule Aachen, Pauwelsstrasse 30, D-5100, Germany*
- A. BOLCK *Faculty of Mathematics and Natural Sciences, University Centre for Pharmacy, University of Groningen, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands*
- P. JANDERA *Department of Analytical Chemistry, University of Pardubice, Faculty of Chemical Technology, Nám. Legií 565, 532 10 Pardubice, Czech Republic*
- P. KURONEN *Department of Chemistry, P.O. Box 6 (Vuorikatu 20), University of Helsinki, FIN-00014 Helsinki, Finland*
- J.J. PESEK *Department of Chemistry, San Jose State University, San José, CA 95192-0101, USA*
- L.C. SANDER *Chemical Science and Technology Laboratory, Organic Analytical Research Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-0001, USA*
- A.K. SMILDE *Laboratory for Analytical Chemistry, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands*
- R.M. SMITH *Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU, UK*
- K. VALKÓ *Department of Physical Sciences, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, UK*
- S.D. WEST *North American Environmental Chemistry Laboratory, Dow-Elanco, P.O. Box 68955, 9410 Zionsville Road, Indianapolis, IN 46268-1053, USA*
- E.J. WILLIAMSEN *Department of Chemistry, San Jose State University, San José, CA 95192-0101, USA*
- S.A. WISE *Chemical Science and Technology Laboratory, Organic Analytical Research Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-0001, USA*

Contents

Chapter 1. Retention prediction based on molecular structure	1
R.M. Smith	
1.1 Introduction	1
1.2 Structure and retention	2
1.2.1 Chromatographic functional group contributions	3
1.2.2 Related prediction studies.....	4
1.3 Functional group effects on retention indices.....	5
1.3.1 Retention prediction based on <i>n</i> -alkanes and <i>n</i> -alkylbenzenes.....	6
1.3.2 Retention prediction based on alkan-2-ones.....	8
1.4 Prediction of retention indices based on alkyl aryl ketones.....	11
1.4.1 Monofunctional compounds	13
1.4.1.1 Aromatic functional groups.....	13
1.4.1.2 Aliphatic functional groups	15
1.4.1.3 Relationship between substituent indices and octanol–water partition substituent increments.....	19
1.4.2 Polyfunctional compounds	21
1.4.2.1 General prediction model.....	23
1.4.2.2 <i>Meta</i> and <i>para</i> groups	29
1.4.2.3 <i>Ortho</i> -substituents	32
1.4.3 CRIPES and expert systems	33
1.4.4 Comparisons with published retention values.....	38
1.5 Other retention index prediction studies.....	40
1.5.1 Retention prediction based on polynuclear aromatic hydrocarbons	41
1.6 Conclusion.....	43
1.7 Acknowledgements.....	43
1.8 Appendix 1.1: Coefficients of regression equations for the effect of eluent on parent, aromatic and aliphatic substituent indices	44
1.9 References	45
 Chapter 2. Retention prediction of pharmaceutical compounds.....	 47
K. Valkó	
2.1 Introduction	47
2.2 Definition and determination of retention.....	48
2.3 Dependence of retention on the column and mobile phase composition	50
2.4 Correlation of retention parameters to the molecular parameters obtained by molecular modelling.....	53
2.5 Retention prediction based on topological matrix and information theory.....	60
2.6 Retention prediction based on the hydrophobicity of drugs	62

2.7	Retention prediction based on empirical increment values.....	68
2.8	Retention prediction based on experimental retention values, thermodynamic considerations with multiparameter approaches	76
2.9	Applications of retention predictions of pharmaceutical compounds	87
2.10	Acknowledgements.....	90
2.11	References	90
Chapter 3.	Retention index scales used in high-performance liquid chromatography	93
	R.M. Smith	
3.1	Introduction	93
3.1.1	Relative retention times	94
3.1.2	Internal and external standards	95
3.1.3	Retention indices	97
3.2	Retention index scales in chromatography	98
3.2.1	Gas chromatography.....	99
3.2.2	Supercritical fluid chromatography	100
3.2.3	Liquid chromatography	103
3.2.4	Micellar electrokinetic chromatography	105
3.3	Retention index scales in high-performance liquid chromatography	107
3.3.1	<i>n</i> -Alkanes.....	107
3.3.2	<i>n</i> -Alkylbenzenes	109
3.3.3	Alkan-2-ones	109
3.3.4	Alkyl aryl ketones.....	111
3.3.5	1-Nitroalkanes	115
3.3.6	Polynuclear aromatic hydrocarbons.....	115
3.3.7	Miscellaneous retention index scales.....	116
	3.3.7.1 Phenolic esters.....	116
	3.3.7.2 Aliphatic esters.....	117
	3.3.7.3 Other retention index scales	118
3.3.8	Comparisons between retention index scales.....	118
3.4	Applications of retention index scales	122
3.4.1	Reproducibility and transferability of retention indices.....	122
3.4.2	Identification	125
3.4.3	Characterization of separation systems.....	125
	3.4.3.1. Column hold-up volume.....	126
	3.4.3.2 Stationary phase characterization	127
	3.4.3.3 Mobile phase characteristics	133
3.4.4	Lipophilicity and biological activity	135
3.4.5	Structure-retention relationships.....	137
3.5	Conclusions	139
3.6	References	140

Chapter 4. Application of retention indices for identification in high performance liquid chromatography	145
R.M. Smith	
4.1 Introduction	145
4.2 Advantages and problems	145
4.3 Pharmaceuticals and toxicological drug samples	147
4.3.1 Toxicological drug analysis	147
4.3.1.1 Studies based on the alkan-2-ones	148
4.3.1.2 Studies based on the alkyl aryl ketones	149
4.3.1.3 Studies based on the 1-nitroalkanes	156
4.3.2 Drug metabolites	156
4.4 Natural products	157
4.4.1 Fungal metabolites	157
4.4.1.1 Mycotoxins	158
4.4.1.2 Other fungal metabolites	159
4.4.2 Plant products	160
4.4.2.1 Spices and flavour components	161
4.4.2.2 Plant toxins	162
4.4.2.3 Gliadins	163
4.4.3 Lichen constituents	163
4.4.4 Other natural products	164
4.5 Environmental samples	164
4.5.1 Chlorinated compounds	164
4.5.2 PAH and aromatic hydrocarbons	165
4.6 Miscellaneous samples	165
4.7 Conclusions	165
4.8 Appendix 1: Reported retention indices in HPLC	166
4.9 References	167
 Chapter 5. Application of nitroalkanes and secondary retention index standards for the identification of drugs	 171
M. Bogusz	
5.1 Introduction	171
5.2 The use of HPLC as a standardized identification method in toxicology	173
5.2.1 Standardization of retention using straight phase silica	173
5.2.2 Standardization of retention using reversed phase silica	175
5.2.2.1. The concept of secondary standards for retention index scale	183
5.2.2.2 Assessment of identification potentials of a HPLC/RI system	199
5.2.2.3 Influence of a biological matrix on the identification potential of a HPLC/RI system	203
5.2.3 Standardization of detection for toxicological screening procedures	204
5.3 References	205

Chapter 6. Identification using retention indices in gradient HPLC.....	209
P. Kuronen	
6.1 Introduction	209
6.2 Problems of reversed-phase columns	210
6.3 Selection of a retention index standard.....	213
6.4 Principles of gradient elution.....	215
6.5 Chromatographic behaviour of retention index standards	217
6.5.1 Isocratic conditions.....	217
6.5.2 Gradient elution.....	219
6.6 Retention indices in qualitative identification	221
6.6.1 Calculation of retention indices.....	221
6.6.2 Reproducibility of gradient retention indices	223
6.6.3 Confirmation of identification	228
6.7 Conclusions	230
6.8 References	230
Chapter 7. Characterization of retention and selectivity in reversed-phase LC using interaction indices.....	235
P. Jandera	
7.1 Introduction	235
7.2 Interaction indices as the descriptors of retention	236
7.2.1 Retention in binary mobile phases.....	238
7.2.2 Retention in ternary mobile phases.....	243
7.3 Calibration of the scale of interaction indices	247
7.4 Prediction of the retention under changing mobile phase composition using interaction indices.....	250
7.5 Interaction indices and the selectivity of separation	253
7.5.1 Non-homologous compounds.....	253
7.5.2 Homologous and oligomeric series.....	255
7.6 Conclusions	263
7.7 Glossary of the terms.....	264
7.8 References	266
Chapter 8. Lipophilic and polar indices	269
P. Jandera	
8.1 Introduction	269
8.2 Retention in homologous series as the basis of lipophilic and polar indices	269
8.3 Molecular structure and lipophilic and polar indices.....	274
8.3.1 Δn_c and Δq indices as the descriptors of the lipophilicity and polarity of solutes	274
8.3.2 Structural contributions to lipophilic and polar indices	276
8.4 Prediction of retention using lipophilic and polar indices	279
8.4.1 Selection of the reference calibration homologous series.....	279
8.4.2 Precision of the predicted retention data	279
8.5 Characterization of selectivity using lipophilic and polar indices	283

8.5.1	Binary mobile phases.....	283
8.5.2	Ternary mobile phases.....	287
8.5.3	Gradient elution.....	290
8.6	Conclusions	292
8.7	Glossary of the terms.....	292
8.8	References	294
Chapter 9.	Solvent selectivity.....	297
	S.D. West	297
9.1	Introduction	297
9.2	Experimental	299
9.2.1	Chemicals and reagents	299
9.2.2	Instrumentation.....	299
9.2.3	Determination of retention data	299
9.2.4	Selection of solvents and solutes	300
9.3	Results and discussion	
9.3.1	Prediction of retention and resolution with steroids	301
9.3.1.1	Retention indices as a function of volume fraction of strong solvent	301
9.3.1.2	Prediction of retention indices for steroids.....	302
9.3.1.3	Prediction of resolution of steroid mixtures	303
9.3.1.4	Optimization of resolution of steroid mixtures.....	307
9.3.1.5	Resolution and the solvent selectivity triangle concept.....	309
9.3.2	Retention and selectivity studies with benzene derivatives	311
9.3.2.1	Retention index variation with solvent selectivity.....	311
9.3.2.2	Resolution and the solvent selectivity triangle	311
9.3.2.3	Prediction of resolution	314
9.3.2.4	HPLC resolution as a function of retention index differences.	314
9.3.2.5	Preadjustment of retention indices for prediction of resolution	318
9.3.3	Reasons for failure of the solvent selectivity triangle	320
9.3.4	Extension of theory to gas chromatography.....	323
9.3.4.1	Prediction of resolution	323
9.3.4.2	McReynolds constants and resolution	324
9.3.4.3	Application of the selectivity triangle to the characterization of GC stationary phase selectivity.....	326
9.3.5	Characterization of RP-HPLC selectivity with adjusted retention indices.....	327
9.3.5.1	Calculation of adjusted retention indices.....	327
9.3.5.2	Probes for characterization of RP-HPLC solvent selectivity...	329
9.3.5.3	Quantitative prediction of resolution with any RP solvent.....	331
9.4	Conclusions	334
9.5	Acknowledgments	335
9.6	References	335

Chapter 10. Retention and selectivity for polycyclic aromatic hydrocarbons in reversed-phase liquid chromatography	337
L.C. Sander and S.A. Wise	
10.1 Introduction	337
10.2 Stationary phase characteristics affecting selectivity in RPLC	338
10.2.1 Phase type	338
10.2.2 Isomer separations	341
10.2.3 Assessing column shape selectivity	343
10.2.4 Pore size effects	346
10.2.5 Bonding density	349
10.2.6 Bonded phase length	350
10.2.7 Mobile phase composition	352
10.2.8 Temperature	353
10.3 Retention indexes	357
10.3.1 Retention index data	358
10.3.2 Length-to-breadth ratio	360
10.3.3 Planar and non-planar PAHs	364
10.3.4 Methyl-substituted PAHs	365
10.4 Summary	368
10.5 References	368
Chapter 11. Comparison of novel stationary phases	371
J.J. Pesek and E.J. Williamsen	
11.1 Introduction	371
11.1.1 Characteristics for the ideal reversed-phase HPLC stationary phase	371
11.2 Characterization techniques	372
11.2.1 Spectroscopic techniques	372
11.2.1.1 IR methods	372
11.2.1.2 NMR methods	374
11.2.1.3 ESCA methods	375
11.2.2 Thermal methods	376
11.2.3 Elemental analytical methods	376
11.2.4 Chromatographic characterization	377
11.3 Monomeric octadecyl silica	378
11.3.1 Separation mechanisms for ODS phases	378
11.3.2 ODS limitations and adjustments	379
11.3.2.1 Different bonded phase-silica linkages	381
11.3.2.2 Differences in ODS columns	383
11.4 Novel silica phases	383
11.4.1 Non-C ₁₈ alkane phases	383
11.4.2 Materials containing other functional groups	385
11.4.2.1 Cyanopropyl-bonded phases	385
11.4.2.2 Amine and phenyl phases	385
11.4.2.3 Non-alkyl phases bonded through a reactive olefin	385
11.4.2.4 Polymer bonded phases	388

11.4.2.5 Liquid crystal phases	388
11.4.3 Chiral phases	390
11.4.3.1 Ligand exchange chromatography	390
11.4.3.2 Direct enantiomeric separation	391
11.4.3.3 Pirkle phases	391
11.4.3.4 Protein phases	392
11.4.3.5 Organometallic phases	393
11.5 Non-silica-based stationary phases	393
11.5.1 Alumina-based stationary phases	393
11.5.1.1 Monomeric alumina phases	393
11.5.1.2 Polymeric alumina phases	395
11.5.2 Carbon-based zirconia	395
11.5.3 Polymer-based stationary phases	397
11.6 Conclusion	399
11.7 References	399
Chapter 12. Multivariate characterization of RP-HPLC stationary phases	403
A. Bolck and A.K. Smilde	
12.1 Introduction	403
12.2 Multivariate characterization	404
12.2.1 Some basic multivariate statistical concepts	405
12.2.1.1 Data matrices; centering and scaling	406
12.2.1.2 Visualization of data	407
12.2.2 Principal component analysis	410
12.2.2.1 The concept of principal component analysis	410
12.2.2.2 The calculation of the principal components	412
12.2.2.3 A least squares interpretation of principal component analysis	414
12.2.3 A principal component example	415
12.2.4 Three-way analysis	417
12.2.5 A three-way analysis example	420
12.3 Marker selection	422
12.3.1 The choice of markers	423
12.3.1.1 The determinant criterion	423
12.3.1.2 The induced variance criterion	423
12.3.1.3 Principal component analysis	424
12.3.1.4 Marker selection and three-way analysis	425
12.3.2 A marker selection example	425
12.4 Predictions	428
12.4.1 Ordinary least squares (OLS)	429
12.4.2 Partial least squares (PLS)	432
12.4.2.1 PLS1	432
12.4.2.2 PLS2	433
12.4.2.3 PLS predictions	434
12.4.3 PLS predictions and marker selection	434

12.4.4 A PLS example.....	435
12.5 Practical examples.....	438
12.5.1 Calibration of octadecyl modified stationary phases of different batches.....	439
12.5.2 Calibration of stationary phases of different types	443
12.6 Appendix A	445
A.1 Rank	445
A.2 Spectral decomposition	446
A.3 The singular value decomposition (SVD).....	446
12.7 References	447
Subject Index	451

CHAPTER 1

Retention prediction based on molecular structure

Roger M. Smith

*Department of Chemistry, Loughborough University of Technology, Loughborough,
Leicestershire, LE11 3TU, UK*

1.1 INTRODUCTION

The retention of a particular analyte in a reversed-phase liquid chromatographic system is dependent on many factors, the structure of the analyte, the nature and chemistry of the stationary phase, the composition of the mobile phase and the temperature. Some of these factors are reasonably well understood, at least on an empirical level, and chromatographers can manipulate eluent composition and even temperature to alter retentions in a predictable manner.

However, the effect of the chemical structure of the analyte on retention is probably the least well described parameter. Most chromatographers recognise the broad influence of polarity and size and their effect on hydrophobicity but not the detailed impact of the addition of a methoxyl, carboxamide or other functional group. Nor in most cases is it possible to predict the composition of the eluent required to result in a predetermined retention (capacity) factor (k). Instead the experimental conditions to achieve a particular retention are usually selected by analogy with related compounds or from experience of analysing a wide range of samples. Most analytical methods in liquid chromatography are then refined on a trial and error basis.

However, in recent years two methods to aid the chromatographer in refining a separation have become available. The first requires no knowledge of the structure of the analyte. A computer programme, often an expert system, uses the retention factors of the components of a mixture from a gradient or isoelutotropic set of separations to propose an eluent mixture, which is predicted to provide optimal resolution or overall run times. These techniques include systems such as Drylab, PESOS, ICOS, and DIAMOND, which are based on prediction and mapping methods, and chemometric techniques, including iteration and Simplex optimization methods. These methods have been well reviewed in recent years [1–3]. Future developments are likely to see the expert systems being sup-

plemented by neural networks, which should enable them to “learn” about the properties of a particular column and instrument, before making their predictions [75].

In most assays the structure of the analyte is known and the second approach has been to predict the retention from the molecular structure. This can be carried out directly by the summation of the retention properties of the structural components or by deriving a physical property, such as the octanol–water distribution coefficient ($\log P$), which can then be related to retention by comparison with analytes of known value [3]. As the structures of any impurities or metabolites in a sample are often known, it should also be possible to predict the optimum conditions for their resolution from the main components. This approach has the potential for true prediction as it can propose initial chromatographic conditions, designed from the start to achieve a particular separation. Two different but closely related aspects of this approach form the subject of this and the following chapters.

The recent literature also includes numerous papers on retention prediction which related retentions under one set of conditions with those using a different proportion of modifier or temperature. For example, changes in retention with mobile phase composition have been recently discussed by Valko *et al.* [4]. A second closely related area has been the selection of robust methods that although they may not be optimized to give the maximum resolution, nevertheless provide methods which are less susceptible to small changes in eluent composition, temperature and or different columns [5,6]. In real life situations this may be an important consideration if the method is to form part of an official method or is required for long-term studies of the stability or quality of a product. Again computer assistance has been provided for the selection of testing conditions and the evaluation of the results.

1.2 STRUCTURE AND RETENTION

The concept that the retention of an analyte in gas or liquid partition chromatography can be expressed as the summation of factors related to its skeleton and individual functional groups was originally proposed by Martin [7]. He suggested that the retention of an analyte can be expressed by the summation of contributions from each of the structural components, alkyl-chains, aromatic rings and functional groups. These substituent values are related to their effects on other equilibria and are recognised as examples of a linear free-energy relationship. The early work in this area on gas-liquid chromatography and thin-layer chromatography have been reviewed by Kaliszan [8,9].

These concepts have led to a wide range of studies, which have examined the effect of the different substituents on the retention of an analyte in liquid chromatography. These quantitative structure–retention relationships (QSRR) studies have encompassed physical properties, topological indices, and additive functions and have been reviewed in detail [8–12]. Similar concepts have long been used for the prediction and calculation of octanol–water partition coefficients ($\log P$) in quantitative structure-activity relationship (QSAR) studies which are important in relating biological activity to structural features. Hansch and Leo [13] have shown that the $\log P$ can be calculated by the summation of a value for a parent compound with contributions for each substituent (π constants) and a