JOURNAL OF CHROMATOGRAPHY LIBRARY - volume 57

retention and selectivity in liquid chromatography

prediction, standardisation and phase comparisons

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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

This book is printed on acid-free paper.

Transferred to digital printing 2006

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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

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R.M. Smith, editor

CHAPTER 1

Retention prediction based on molecular structure

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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 isoeluotropic 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-

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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 a 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