HIGH-PERFORMANCE
GRADIENT ELUTION
The Practical Application of the Linear-Solvent-Strength Model

LLOYD R. SNYDER
LC Resources, Inc., Orinda, California

JOHN W. DOLAN
LC Resources, Inc., Amity, Oregon
HIGH-PERFORMANCE
GRADIENT ELUTION
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... every natural science involves three things: the sequence of phenomena on which the science is based [experimental observation]; the abstract concepts which call these phenomena to mind [a model]; and the words in which the concepts are expressed [the present book].

Antoine Laurent Lavoisier [with parenthetical additions by the authors], *Traité Elémentaire de Chemie* (1789)
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High-performance liquid chromatography (HPLC) is today widely used for separation and analysis [1, 2]. Many samples cannot be successfully separated by the use of fixed (isocratic) conditions, but instead require gradient elution (also called solvent programming): a change in mobile phase composition during the separation, so as to progressively reduce sample retention. To take full advantage of such gradient-HPLC separations, the user needs an understanding of gradient elution comparable to that required for isocratic separation. Our reference in the present book to high-performance gradient elution implies such an understanding, accompanied by the use of state-of-the-art equipment, columns and experimental technique. Because of the major importance of separations by reversed-phase liquid chromatography (RP-LC), this separation mode will be assumed unless otherwise stated (Sections 6.2.2, 8.2, and 8.3 discuss gradient elution with ion-exchange and normal-phase chromatography).

Several previous reviews or books ([3–8] and Chapter 8 of [2]) discuss the principles and practice of gradient elution, as these were understood at the time these accounts were written. However, these past reviews now appear dated, incomplete, and/or unnecessarily complicated for practical application. Hence the present book has been written with three different goals in mind: (a) a practical summary of what the reader needs to know in order to carry out any gradient separation; (b) a conceptual understanding of how gradient elution works; and (c) a detailed examination of the underlying theoretical framework of gradient elution, for application to special situations and to satisfy any lingering doubts of the reader. Because many readers will be interested in simply using gradient elution or developing a gradient procedure, this application is emphasized in the present book.

Of the various ways in which chromatography is applied today, few have been as misunderstood as the technique of gradient elution, which for some continues as “a riddle wrapped in a mystery inside an enigma” [9]. “Simple” isocratic separation can itself be a challenge, while gradient elution involves added complexity in terms of equipment, procedures, the interpretation of results, and a preferred method development strategy. Compared with isocratic separation, gradient elution is also regarded as (a) subject to more experimental problems and (b) inherently slower and less robust, as well as (c) presenting special difficulty for method transfer from one laboratory to another. Because of these potentially unfavorable characteristics of gradient elution, many workers in the past have avoided its use where possible. It is a premise of the present book that gradient elution can be much less hard to understand and much more easy to use than has been assumed previously.

Gradient elution sometimes appears to contradict our prior experience based on isocratic separation. In isocratic elution, for example, a reduction in flow rate by a factor of 2, or a 2-fold increase in column length, leads to a doubling of
retention times and a 1.5- to 2-fold increase in peak widths. Similar changes in flow rate or column length when using gradient elution usually result in much smaller variations in peak retention or width. In isocratic elution, a change in flow rate or column length also has no effect on the relative spacing of peaks within the chromatogram. However, this is often not the case for gradient elution; indeed, such “surprises” are inherent in its nature. Changes in retention times and sample resolution, when flow rate, column length, or gradient time is varied in gradient elution, also depend on the nature of the sample being separated. In the latter connection, it is important to recognize four different sample groupings or classifications: “regular”/low-molecular-weight, “regular”/high-molecular-weight, “irregular”/low-molecular-weight, and “irregular”/high-molecular-weight samples. The significance for gradient elution of each of these four sample types is examined in this book. Except in Chapter 6, however, we will assume “low-molecular-weight” samples with molecular weights <1000 Da.

The essential similarity of isocratic and gradient elution is often overlooked, but once recognized it allows a much easier understanding of gradient separation, as well as an “intuitive” feeling for what will happen when some change in gradient conditions is made. In this book, we will use the linear-solvent-strength (LSS) model of gradient elution [3, 5, 7] as a bridge between separations by isocratic and gradient elution. This model also leads to near-exact equations for retention time, peak width, and resolution as a function of gradient conditions, as well as the widespread implementation of computer simulation as an aid to HPLC method development. For any sample, data from two or more experimental gradient runs can be used by the computer to predict either isocratic or gradient separation as a function of conditions, thereby facilitating the systematic improvement of the separation. Computer simulation is especially useful for developing gradient methods, and it has been used extensively in the present book as a means of more effectively illustrating the effects of different experimental conditions on gradient separation. It is also our hope that this book can prove useful “in reverse,” whereby a better understanding of gradient elution may even improve our application of isocratic separation.

The beginning of the book (Chapter 1, Section 2.1, and Chapter 3) describes the application of isocratic and gradient elution for typical samples (those with molecular weights <1000 Da), with minimal digression into the derivations of important equations and little attention to less important aspects of gradient elution. Sections 2.2–2.4 provide a conceptual basis for the better interpretation and use of gradient elution, which some (but probably not all) readers will want to read prior to Chapter 3. In Chapter 4, the equipment required for gradient elution is discussed. Chapter 5 deals with experimental problems that can be encountered in gradient elution as well as related troubleshooting information. Chapter 6 recognizes important differences in gradient elution when this technique is used for macromolecular samples, for example, large peptides, proteins, nucleic acids, viruses, and other natural or synthetic polymers. Chapter 7 expands the discussion of earlier chapters to the use of gradient elution for preparative separations, that is, the injection of larger samples for recovery of purified material. Chapter 8 examines (a) separations which feature the combination of gradient elution with mass spectrometric detection.
(LC-MS), (b) the application of gradient elution to normal-phase and ion-exchange separations, and (c) the use of complex gradients formed from three or more solvents. Chapter 9 concludes with a more detailed treatment of the fundamental equations of gradient elution, including attention to so-called “nonideal” contributions to gradient separation.

The present book assumes some familiarity with the principles and practice of HPLC [2]. For a quick and practical summary of the essentials of gradient elution separation, it is suggested that the reader read Chapter 1, Section 2.1, Chapter 3, and Chapter 4, in this order, then consult Chapter 5 (Troubleshooting) as needed. If greater insight into how gradient elution works is desired, Sections 2.2–2.4 provide additional background, with further detail available in Chapter 9. Biochemists may want to start with Chapters 1 and 3, plus Section 6.2, while workers engaged in the isolation of purified sample components will benefit especially from Chapter 7 (Preparative Separations). A “reading plan” for the book is suggested by Figure P.1, with the bold topics comprising a minimal introduction to gradient elution.

No profit grows, where is no pleasure taken; In brief, sir, study what you most affect.

—William Shakespeare, The Rape of Lucrece

The present book is heavily cross-referenced to other sections of the book, so as to allow the reader to follow up on topics of special interest, or to clarify questions that may arise during reading. Because extensive cross-referencing represents a potential distraction, in most cases it is recommended that the reader simply ignore these invitations to jump to other parts of the book. Some chapters include parts that are of greater academic than practical interest; these sections are in each case clearly identified (introduced with an advisory in italics), so that they can be bypassed at the option of the reader. We have also taken pains to provide definitions for all symbols used in this book (Glossary section), as well as a comprehensive and detailed index.

For the past 30 years, gradient elution has been a major research focus for us. During this time, we have worked together to better understand and apply this powerful experimental procedure, and we have also created commercial software (DryLab®) for the more efficient use of gradient elution by numerous workers throughout the world (“computer simulation”). For one of us (LRS), an interest in this topic extends back another 15 years into the early 1960s. The present book therefore represents the culmination of an interest of long standing, as well as an attempt at a complete and detailed account of the subject. We hope that the book will find use by practical workers throughout the world. During the past 35 years, another scientist, Pavel Jandera from the University of Pardubice, has similarly devoted much of his career to the study and elucidation of the principles and practice of gradient elution. The present book owes much to his many contributions in this area, which did not stop with the publication of his book on gradient elution in 1985 [6] or his recent review of the subject [8].

We very much appreciate the assistance of four co-authors, who were responsible for the preparation of Sections 6.2.2.4 [Carl Scandella (Carl Scandella
Consulting, Bellevue WA), Paul Shabram (Ventana Biosciences, San Diego, California), and Gary Vellekamp (Schering Plough Research Institute, Union, New Jersey) and 7.4 [Geoff Cox (Chiral Technologies, Inc., West Chester, Pennsylvania)]. We are likewise grateful to a number of past collaborators who have greatly assisted our own research on gradient elution: Geoff Cox, Pete Carr, Julie Eble, Russel Gant, Barbara Ghrist, Jack Kirkland, Tom Jupille, Dana Lommen, Dan Marchand, Imre Molnar, Thomas Mourey, Hans Poppe, Mary Ann Quarry, Bill Raddatz, Dennis Saunders, Marilyn Stadalius, Laurie Van Heukelem, Tom Waeghe, and Peng-Ling Zhu. Finally, we very much appreciate the dedicated efforts of several reviewers of this book prior to its publication: Geoff Cox, John Ford, Pavel Jandera, Tom Jupille, John Kern, James Little, Dan Marchand, Jim Merdink, Tom Mourey, Uwe Neue, Carl Scandella, Peter Schoenmakers, Mark Stone, Tim Wehr, Loren Wrisley, Patrick Lukulay, and Jianhong (Jane) Zhao. Several of the latter reviewers have provided further assistance by supplying preprints or reprints of their own work.
REFERENCES


LLOYD R. SNYDER
JOHN W. DOLAN

Orinda, California
Amity, Oregon
September 2006
Glossary of Symbols and Terms

This section is divided into “Major symbols” and “Minor symbols.” “Minor symbols” refer to symbols that are used only once or twice. Most symbols of interest will be included in “Major symbols.” Equations which define a particular symbol are listed with that symbol; for example, “Equation (2.18)” refers to Equation (2.18) in Chapter 2. The units for all symbols used in this book are indicated. Where IUPAC definitions or symbols differ from those used in this book, we have indicated the corresponding IUPAC term (from ASLDID 009921), for example, $t_M$ instead of $t_0$.

**Major Symbols and Abbreviations**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>column hydrogen-bond acidity; Appendix III</td>
</tr>
<tr>
<td>A solvent</td>
<td>mobile phase at the start of the gradient</td>
</tr>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>$b$</td>
<td>intrinsic gradient steepness; Equation (2.11) (see discussion in Section 1.3.3)</td>
</tr>
<tr>
<td>B, B solvent</td>
<td>mobile phase at the end of the gradient; percentage B refers to the volume-percent of B in the mobile phase</td>
</tr>
<tr>
<td>$B$</td>
<td>column hydrogen bond basicity; Appendix III</td>
</tr>
<tr>
<td>$C$</td>
<td>column cation exchange capacity; Appendix III</td>
</tr>
<tr>
<td>$C$</td>
<td>concentration of the salt counter-ion in IEC (assuming a univalent counter-ion)</td>
</tr>
<tr>
<td>$C^*o$, $(C)_{o}$</td>
<td>value of $C$ in gradient elution (for band at column midpoint)</td>
</tr>
<tr>
<td>$(C)<em>{f}$, $(C)</em>{f}$</td>
<td>values of $C$ at beginning (o) and end (f) of gradient</td>
</tr>
<tr>
<td>$d_p$</td>
<td>particle size ($\mu$m)</td>
</tr>
<tr>
<td>$F$</td>
<td>flow rate (mL/min)</td>
</tr>
<tr>
<td>$G$</td>
<td>gradient compression factor; Equation (9.36)</td>
</tr>
<tr>
<td>$G_{12}$</td>
<td>ratio of peak widths before and after passage of a step-gradient through a band within the column; $= W_2/W_1$ in Figure 9.4</td>
</tr>
<tr>
<td>GLP</td>
<td>good laboratory practice</td>
</tr>
<tr>
<td>$H$</td>
<td>plate height (mm); Equation (9.58)</td>
</tr>
</tbody>
</table>
GLOSSARY OF SYMBOLS AND TERMS

H
column hydrophobicity; Appendix III
HIC
hydrophobic interaction chromatography
HILIC
hydrophilic interaction chromatography
HPLC
if you need to look up the meaning of HPLC, this is the wrong book for you
i.d.
column internal diameter (mm)
IEC
ion-exchange chromatography
IQ
installation qualification; Section 5.1.1.1
k
isocratic retention factor; Equation (2.4) (formerly called capacity factor, $k'$)
$k^*$
gradient retention factor; equal to value of $k$ for a band when it reaches the column mid-point; Equation (2.13), Figure 1.7 (previously, a different symbol was used, $\tilde{k}$)
$k^*(a), k^*(b), \ldots$ value of $k^*$ for peak $a$, $b$, and so on
$k_c$
value of $k$ at elution; Figure 1.7
$k_i, k_j, \ldots$ value of $k$ for peaks $i$, $j$, etc. Also, $k_i$ is the instantaneous value of $k$ for a band at any time during its migration through the column; Equation (9.1)
$k_0$
the value of $k$ in gradient elution at the start of the gradient [Equation (2.10)]; also (Chapter 7 and Appendix V only), the value of $k$ in isocratic elution for a small weight of injected sample (in distinction to the value of $k$ for a large sample)
$k_w$
value of $k$ for water or 0 percent B as mobile phase ($\phi_0$) (extrapolated value)
$k_1, k_2, \ldots$ value of $k$ for solute 1, 2, and so on; also, value of $k$ for two different values of $\phi$ ($\phi_1$ and $\phi_2$)
$L$
column length (mm)
LC
liquid chromatography
LCCC
liquid chromatography under critical conditions
LC-MS
liquid chromatography–mass spectrometry (Section 8.1)
LC-MS/MS
LC-MS with triple quadrupole mass spectrometer (Section 8.1)
LSS
linear-solvent-strength (model) (Sections 1.4.2, 9.1)
m
stoichiometry factor in NPC [Equation (8.8)]; also, $|z|$ in IEC
$M$
solute molecular weight; also counter-ion molarity in IEC
MeOH
methanol
MS
mass spectrometric
$n$
number of peaks in a chromatogram or sample; also the designation of the $n$th oligomer in an oligomeric sample
$N$
column plate number (isocratic); Equation (2.5); also native protein in Figure 6.4
GLOSSARY OF SYMBOLS AND TERMS

$N_0$ column plate number for a small weight of sample; Equation (7.3)
$N^*$ column plate number (gradient); Equation (2.20)
NPC normal-phase chromatography
ODS octdecylsilyl; $C_{18}$
OQ operational qualification; Section 5.1.1.1
$p$ quantity used to calculate gradient compression factor $G$; Equation (9.35)
$P$ column pressure-drop (psi); MegaPascals (MPa $= 145$ psi) is also commonly used, but not in the present book (the IUPAC symbol is $\Delta p$)
$PC$ peak capacity; the number of peaks with $R_s = 1$ that can be fit into a given chromatogram; see Figure 2.11(a) and related text.
$PC_{req}$ required peak capacity for the separation of a sample containing $n$ components; see Figure 2.11(c) and related text (previously defined as “$PC^*$”)
prep-LC preparative liquid chromatography; Chapter 7
psi pounds per square inch; see $P$
PQ performance qualification; Section 5.1.1.1
QC quality control
$r$ fractional migration of a band through the column during gradient elution; Equation (9.12)
$R$ equal to $1/(1 + k)$ (the IUPAC symbol is $\kappa$)
$R_1, R_2$ equal to $R$ for peaks 1 and 2
RP-LC reversed-phase liquid chromatography
$R_F$ fractional migration of a peak through the column after the passage of one column-volume $V_m$ of mobile phase through the column; $R_F = 1/(1 + k)$
$R_s$ resolution of two adjacent peaks; Equation (2.6), Figure 2.1; also see Equations (2.8) (isocratic) and (2.21) (gradient); “critical” resolution refers to the value of $R_s$ for the least well separated pair of peaks in a chromatogram
$S$ constant in Equation (1.2) for a given solute and experimental conditions; equal to $d(\log k)/d\phi$
$S$ column steric resistance to penetration; Appendix III
SA surface area ($m^2$); Equation (7.5)
time after the beginning of a gradient run (min); Equation (9.2); also, time after the end of a gradient run (Fig. 9.5a)
T-P “touching-peak”; preparative separation in which a large enough sample is injected to allow the desired product peak to touch an adjacent peak in the chromatogram (Section 7.1)
TFA  trifluoroacetic acid
THF  tetrahydrofuran
$ t_D $  system dwell time (min); equal to $ V_D/F $
$ t_{delay} $  gradient delay time (min), corresponding to initial isocratic elution before the start of the gradient
$ t_{eq} $  equilibration time for inter-run column equilibration in gradient elution (min); equal to $ V_{eq}/F $
$ t_G $  gradient time (min)
$ t_0 $  column dead time (min); retention time of an unretained peak such as thiourea (the IUPAC symbol is $ t_M $)
$ t_R $  retention time (min); see Figure 2.1 and related text
$ t_{R,a}, t_{R,b}, $ etc.  values of $ t_R $ for peaks $ a, b, $ etc.
$ (t_R)_{avg} $  average value of $ t_R $; Figure 3.2
$ t'_R $  corrected retention time, equal to $ t_R - t_0 $
ULOQ  upper limit of quantification
USP  $ United States Pharmacopeia $  
UV  ultraviolet
$ V $  volume of mobile phase that has entered the column by a given time (mL); Equation (9.1)
$ V_D $  equipment dwell volume (mL); volume of system flowpath between inlet to gradient mixer and column inlet
$ V_{eq} $  equilibration volume (mL) of A solvent used for inter-run column equilibration in gradient elution
$ V_m $  column dead volume (mL); $ V_m = t_0F $; unless noted otherwise, a column internal diameter of $ d_c = 4.6 $ mm is assumed, in which case $ V_m \approx 0.01L $, where $ L $ is column length in mm. Otherwise, $ V_m \approx 0.0005(\text{column i.d.)}^2 L $, where column i.d. and $ L $ are in mm (the IUPAC symbol is $ V_M $)
$ V_M $  the “mixing volume” of the gradient system (mL); Table 9.2
$ V_R $  retention volume (mL); $ V_R = t_R F = V_m(1 + k) $
$ V'_R $  corrected retention volume (mL), equal to $ V_R - V_m $
$ V_s $  sample volume (mL)
$ W $  baseline peak width (min); Figure 2.1 (IUPAC symbol is $ W_b $)
$ W_i, W_j, $ etc.  value of $ W $ for peaks $ i, j, $ etc.
$ W_0 $  value of $ W $ for a small sample; Equation (7.2)
$ w_s $  column saturation capacity (mg)
$ W_{th} $  contribution to $ W $ from a sufficiently large sample weight (min); Equation (7.2)
\( w_x \) injected weight of compound \( x \) (mg)

\( W_{1/2} \) peak width at half height; Figure 2.1 (the IUPAC symbol is \( W_h \))

\( x \) fractional migration of a solute band through the column (Figure 1.7); also, band width in Figure 9.3

\( x_i, x_j \) values of \( x \) for solutes \( i \) and \( j \)

\( z \) effective charge on a sample compound in IEC

\( \alpha \) selectivity factor (isocratic); Equation (2.8)

\( \alpha^* \) selectivity factor (gradient) when the band-pair is at the column midpoint

\( \alpha_0 \) the value of isocratic \( \alpha \) or gradient \( \alpha^* \) for a small sample

\( \beta \) equal \( t_{G1}/t_{G2} \); Equation (9.48)

\( \delta t_R \) a change in retention time \( t_R \) due either to incomplete column equilibration or solvent demixing; also, an error in a calculated value of \( t_R \); Equation (9.43)

\( \delta\delta t_R \) difference in \( \delta t_R \) for two adjacent peaks

\( \Delta t_R \) difference in retention times for two peaks (min), for example, Equation (2.24a), Figure 3.2

\( \delta\phi \) error in calculated value of \( \phi \) at elution; Equation (9.43)

\( \delta\phi_m \) distortion of the gradient as a result of gradient rounding; Figure 9.7(a)

\( \Delta\phi \) gradient range, equal to the final value of \( \phi \) in the gradient (\( \phi_f \)) minus the initial value (\( \phi_0 \))

\( \phi \) volume fraction of B solvent in the mobile phase; equal to 0.01 times percentage B

\( \phi_c \) value of \( \phi \) for “critical elution behavior”

\( \phi_e \) value of \( \phi \) for mobile phase at the time a band elutes from the column

\( \phi_t \) value of \( \phi \) for mobile phase at end of gradient; for example, for 10–80 percent B gradient, \( \phi_t = 0.80 \)

\( \phi_0 \) value of \( \phi \) for mobile phase at start of gradient; for example, for 10–80 percent B gradient, \( \phi_0 = 0.10 \)

\( \phi^* \) value of \( \phi \) for mobile phase when a band is at the column mid-point

\( \eta \) solvent viscosity (cPoise); Table IV.1 of Appendix IV

2-D two-dimensional

The Jandera and Schoenmakers groups (and some other workers) have used different symbols than those employed in this book and by the authors in previous publications. Equivalent terms for these different groups are as follows.
**MINOR SYMBOLS**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>A_{HIC}</td>
<td>d(log ( k ))/d(C_{AS}); Equation (6.7)</td>
</tr>
<tr>
<td>API</td>
<td>atmospheric pressure ionization (includes APCI and ESI)</td>
</tr>
<tr>
<td>amu</td>
<td>atomic mass unit; equal to 1 Da</td>
</tr>
<tr>
<td>APCI</td>
<td>atmospheric pressure chemical ionization interface</td>
</tr>
<tr>
<td>ASF</td>
<td>peak asymmetry factor</td>
</tr>
<tr>
<td>AU</td>
<td>absorbance units</td>
</tr>
<tr>
<td>( b_A, b_Z )</td>
<td>value of ( b ) for first peak ( A ) and last peak ( Z ) in the chromatogram [Equations (2.23) and (2.23a)]</td>
</tr>
<tr>
<td>BA</td>
<td>benzyl alcohol; Figure 7.13</td>
</tr>
<tr>
<td>( b^* )</td>
<td>designation of a compound in Figure 7.12</td>
</tr>
<tr>
<td>C</td>
<td>( p )-cresol; Figure 7.13</td>
</tr>
<tr>
<td>C_{AS}</td>
<td>concentration of ammonium sulfate in HIC; Equation (6.7)</td>
</tr>
<tr>
<td>D</td>
<td>fully denatured protein native protein; Figure 6.4</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton; equal to 1 amu</td>
</tr>
<tr>
<td>( d_c )</td>
<td>column internal diameter (mm)</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization interface (for MS)</td>
</tr>
<tr>
<td>( E_{T(30)} )</td>
<td>measure of mobile phase polarity derived from spectroscopic measurements; Equation (9.51)</td>
</tr>
<tr>
<td>( F_s )</td>
<td>column-matching function; Equation III.1 of Appendix III</td>
</tr>
<tr>
<td>( h )</td>
<td>peak height (relative units); Figure 2.1; also, reduced plate height; Equation (9.56)</td>
</tr>
<tr>
<td>( h_{1/2} )</td>
<td>one half of peak height; Figure 2.1</td>
</tr>
<tr>
<td>( H_0 )</td>
<td>value of ( H ) for a small sample; Equation (V.4) of Appendix V</td>
</tr>
<tr>
<td>( H_{th} )</td>
<td>contribution to ( H ) of a large sample; Equation (V.2) of Appendix V</td>
</tr>
<tr>
<td>( K )</td>
<td>equilibrium constant for solute retention</td>
</tr>
<tr>
<td>( k_{ACN} )</td>
<td>value of ( k ) for pure ACN as mobile phase; Equation (6.17)</td>
</tr>
</tbody>
</table>
$k_{\text{H2O}}$ value of $k$ for water as mobile phase in HILIC; Equation (6.14)

$k_i, k_j$ value of $k$ for peaks $i$ and $j$, respectively

$k_{o,A}, k_{o,Z}$ value of $k_o$ for first peak $A$ and last peak $Z$ in the chromatogram [Equations (2.23) and (2.23a)]

$k_{wi}, k_{wj}$ value of $k_w$ for peaks $i$ and $j$

$k_0$ value of $k$ for $C_{\text{AS}} = 0$ in HIC [Equation (6.7)]

$k_{2.5}$ the value of $k$ for 2.5 M ammonium sulfate in HIC; Equation (6.8)

LLE liquid–liquid extraction

$m_{\text{HILIC}}$ $d(\log k)/d(\log \phi_{\text{H2O}})$ in HILIC; Equation (6.14)

MRM multiple reaction monitoring (MS/MS; Section 8.1)

MSD mass selective detector; single-quadrupole mass spectrometer

MTBE methyl-$t$-butylether

$m/z$ mass-to-charge ratio

P phenol; Figure 7.13

PD partially denatured protein; Figure 6.4

PE 2-phenylethanol; Figure 7.13

PEEK poly-ether-ether-ketone; plastic tubing used for HPLC connections

$p, q$ constants in Equation (6.19)

rhGH recombinant human growth hormone

SC standard calibrator

$S_{\text{HIC}}$ equal to $-2.5 A_{\text{HIC}}$ in HIC; Equation (6.8)

$S_i, S_j$ value of $S$ for peaks $i$ and $j$

SIM selective ion monitoring; also single ion monitoring (MS)

SPE solid-phase extraction

t_{G1}, t_{G2}, \text{etc.} values of $t_G$ for runs 1, 2, and so on

t_{R(1)}, t_{R(2)} retention times of peaks 1 and 2, respectively (min)

t_{R,A}, t_{R,Z}$ values of $t_R$ for first peak $A$ and last peak $Z$ in the chromatogram (min)

$W_b$ value of $W$ for peak $b$

$W_i, W_j$ baseline peak widths of peaks $i$ and $j$, respectively (min)

$w_{\text{ion}}$ “loading function” in prep-LC; Equation (V.3)

$\delta k$ error in calculated value of $k$ at elution; Equation (9.46)

$\Delta x$ fraction of a column length; Equation (9.19), Figure 9.2

$\phi_{A}, \phi_{B}, \phi_{AB}$ values of $\phi$ for the mobile phase in reservoir $A$, $B$ and a mixture of $A$ and $B$ where the volume fraction of $A$ is $\phi_{AB}$ (Section 1.3)

$\phi_{\text{HIC}}$ defined as $-(C_{\text{AS}} - 2.5)/2.5$; Equation (6.8)
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi_{\text{H2O},f}$, $\phi_{\text{H2O},o}$</td>
<td>value of $\phi_{\text{H2O}}$ at beginning (o) and end (f) of a HILIC gradient</td>
</tr>
<tr>
<td>$\sigma_g$</td>
<td>surface area per unit weight of column packing ($\text{m}^2/\text{g}$); Equation (7.5)</td>
</tr>
<tr>
<td>$v$</td>
<td>reduced velocity; Equation (9.57)</td>
</tr>
<tr>
<td>$\psi$</td>
<td>phase ratio (the IUPAC symbol is $\beta$)</td>
</tr>
</tbody>
</table>