

Separation Science 'MS Solutions' is the premier online resource for analytical scientists working with mass spectrometry across Europe, the USA and the Middle East. Covering MS method fundamentals, practicalities and troubleshooting it offers chromatographers and analytical chemists a genuine e-learning platform and searchable archive resource.

Tech Tip

Peptide Sequencing with Electrospray LC/MS Part 1: Ion Types and Nomenclature



One of the most significant and important applications for mass spectrometry is the sequencing of polypeptides by electrospray LC/MS. An error in the sequence or the substitution of one amino acid with another can completely alter the biological function of a peptide molecule. Determination of sequences is therefore a vital part of biomedical research, proteomics, and the manufacture of peptide-based drug substances.

[Click here to read more...](#)



Featured Applications

Comprehensive analysis of crude oil by two-dimensional GC (GCxGC) and time-of-flight (TOF) MS

Food Safety Analysis: LC/MS/MS Applications Using Core-Shell Technology HPLC Columns

Non-targeted Screening and Accurate Mass Confirmation of 510 Pesticides Using High Resolution Benchtop LC/MS

[Click titles to learn more](#)



Frederick Klink

Peptide Sequencing with Electrospray LC/MS

Part 1: Ion Types and Nomenclature

One of the most significant and important applications for mass spectrometry is the sequencing of polypeptides by electrospray LC/MS. An error in the sequence or the substitution of one amino acid with another can completely alter the biological function of a peptide molecule. Determination of sequences is therefore a vital part of biomedical research, proteomics, and the manufacture of peptide-based drug substances. We will discuss the basics of peptide sequencing with mass spectrometry in the next three issues of *MS Solutions*.

The sequence of a polypeptide is determined by subjecting the ionized peptide to fragmentation by collisionally activated dissociation (CAD) in an MS/MS instrument. Peptides are introduced through an electrospray interface and are protonated to form positive ions of the type $[M+nH]^{+n}$ where $n \geq 1$. These ions are selected as MS/MS precursors. A wide variety of product ions may be formed because of bond cleavages along the polypeptide backbone or partial to complete cleavage of amino acid side-chains. Those bond cleavages which occur along the peptide

backbone are the most important for sequence determination, particularly the *b* and *y* ions which result from cleavage of peptide bonds. The neutral losses resulting from formation of *b* and *y* ions represent amino acid residue masses and this simplifies interpretation of the MS/MS spectra.

Figure 1 shows the nomenclature which has been adopted for naming the ions formed from peptide backbone cleavages. The vertical lines show the bond cleavage and the arrows point to the product ion, the remaining portion of the precursor being the neutral loss. The product ions

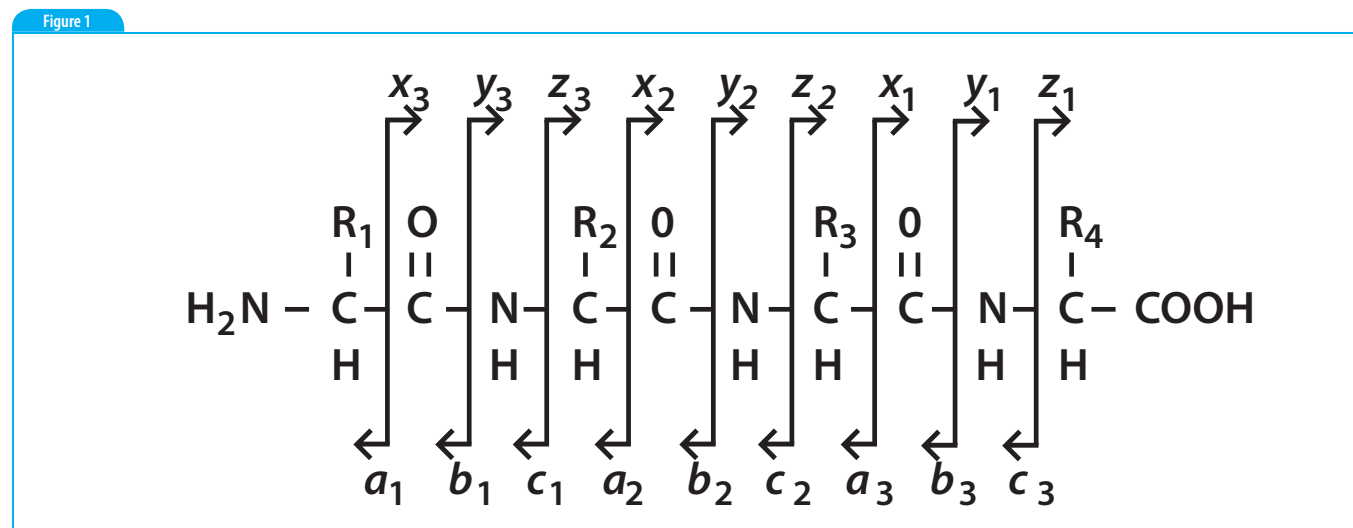


Figure 1: Nomenclature for naming the ions formed from peptide backbone cleavages.

Figure 2

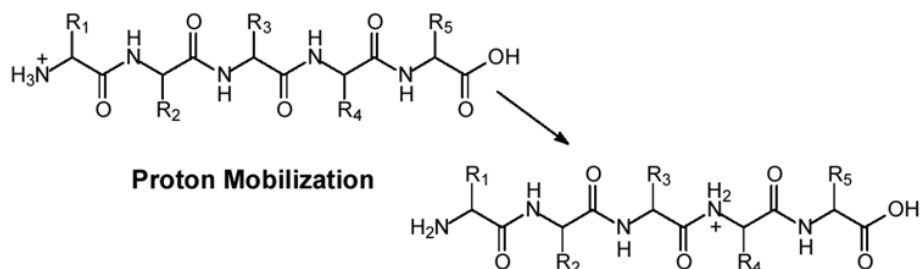


Figure 2: The concept of a 'mobile proton'.

are referred to by the letters *a*, *b*, *c* and *x*, *y*, *z*. The *a*, *b*, *c* series of ions refer to the charge remaining with the N-terminal portion of the peptide ion while the portion containing the C-terminus is the neutral loss. Peptide sequences are, by convention, listed from the N- to the C-terminus, therefore the choice of the *a*, *b*, *c* nomenclature is mnemonic as these are the first three letters of the alphabet. Similarly, the *x*, *y*, *z* ions are those product ions in which the charge is retained on the C-terminal portion of the ion.

A subscript is added to the product ion letter designation to indicate the number of amino acid residues present in that product ion. For example, the a_3 ion in the figure retains three amino acid residues from the intact precursor ion. The y_1 ion retains only a single residue.

Note also in Figure 1 that a single bond cleavage may result in the formation of two different ions. For example, in the figure, if we look to the cleavage of the peptide bond between residues 2 and 3 we see that this cleavage may result in a b_2 ion or a y_2 ion depending on which portion of the precursor ion retains the charge. The mechanism for this type of ion formation is shown in Figure 2.

Figure 2 illustrates the concept of a 'mobile proton' meaning that for a single charge peptide ion the proton might reside on any of the amide nitrogens or on the N-terminal amine. The probability of a proton adducting to a given nitrogen in the peptide is largely dependent on the amino acids present in the sequence and this, in turn, leads to CAD fragmentation patterns being sequence-dependent — more about this in a future installment of *MS Solutions*.

Figure 3

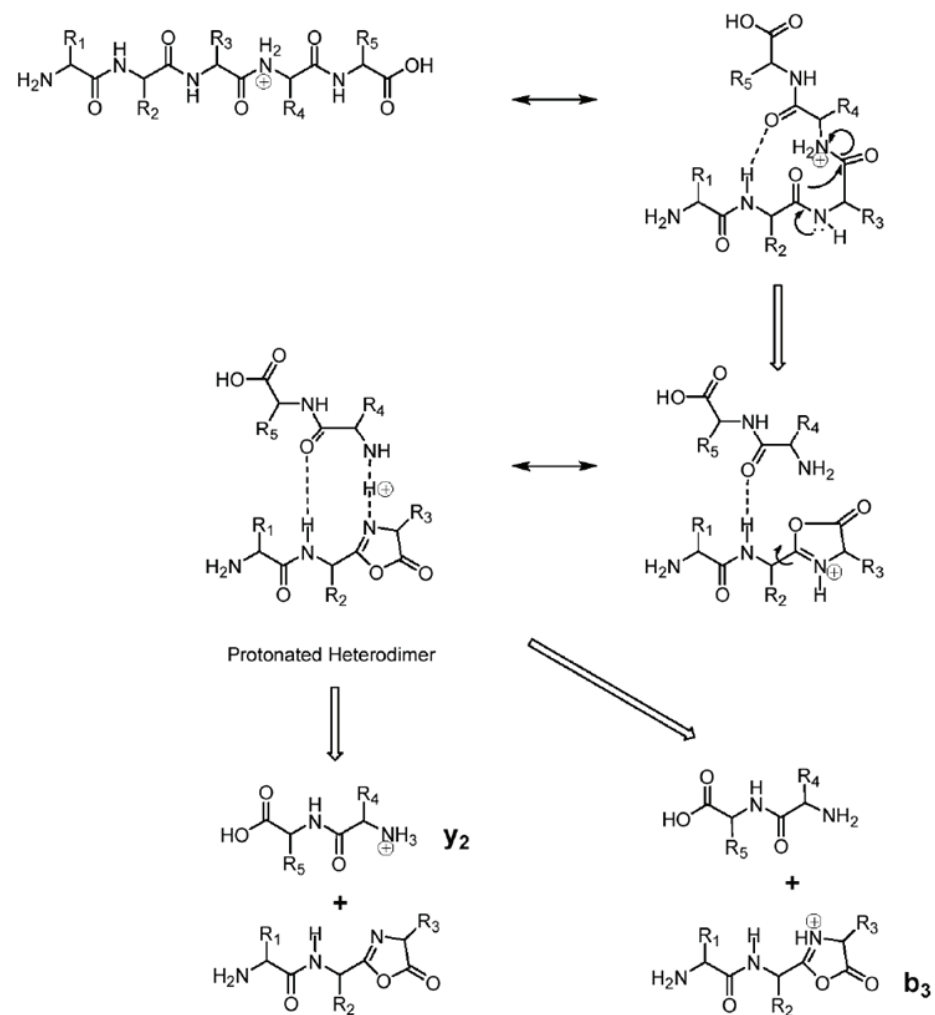
Figure 3: Mechanism for formation of both *b* and *y* ions.

Figure 3 shows a mechanism for formation of both *b* and *y* ions from the same bond cleavage. Under CAD conditions, electrons move in response to the charge resulting in cleavage of the peptide bond proximal to the charge, i.e., between residues 3 and 4. The intermediate species in this



SEPARATION SCIENCE ASIA

New Horizons in Separation and Detection Techniques

2011

27-28 July, 2011, Biopolis, Singapore

Separation Science Asia 2011 offers a world-class educational experience for analytical chemists working across pharmaceutical, food, environmental, clinical, bioscience and energy industries. A premier invited speaker list has been put together, which will provide delegates with the opportunity to learn from and interact with some of the leading exponents of separation techniques from across the globe. Key invited presentations include:

High Throughput Sample Preparation Based on Solid Phase Microextraction

Janusz Pawliszyn (University of Waterloo, Canada)

HPLC Troubleshooting: Listen to the Chromatogram

John W. Dolan (LC Resources, USA)

Miniaturized Sample Preparation for GC Analysis of Volatile Organic Compounds

Yoshihiro Saito (Toyohashi University of Technology, Japan)

Improved Separation Performance and Detection Sensitivity of Complex Samples with Novel Materials

Hanfa Zou (Dalian Institute, China)

"Just Enough" Sample Preparation: A New Trend in Sample Handling

Ron Majors (Agilent Technologies, USA)

Practical Ways to Reduce GC Analysis Time

Jaap de Zeeuw (Restek, The Netherlands)

Computer-Assisted Method Development in Ion Chromatography

Paul Haddad (University of Tasmania, Australia)

Novel Monolithic and Packed Columns for HPLC Applications

Haifei Zhang (University of Liverpool, UK)

MD-SPE: A Magic Bullet for Automated Clean-up of Biofluids and Undisturbed LC-MS/MS Analysis of Drugs

Karl-Siegfried Boos (Medical Center of the University of Munich, Germany)

Advances and New Approaches in Modern Liquid Chromatography

Hernan Cortes (H.J. Cortes Consulting, LLC)

Designing a New Concept HPLC Column

Andrew Shalliker (University of Western Sydney, Australia)

Rapid Analysis of Plants by Ambient MS and On-Chip Sample Clean-up

Teris A. van Beek (Wageningen University, The Netherlands)

Dried Blood Extracts (DBE): An Attractive Alternative to Dried Blood Spots (DBS)

Rosa Morello (Medical Center of the University of Munich, Germany)

Production and Characterization of Polymer Monoliths: Trials and Tribulations

Brett Paull (Dublin City University, Ireland)

+20 additional speakers and topics -

Click here to see more>>

[More Information](#)

[Register](#)

CALL FOR PAPERS

Abstract submission is now available for Separation Science Asia 2011. The initial deadline for abstracts is 29 April, 2011.

Abstracts are welcome on all aspects of chromatography, electrophoresis, mass spectrometry and sample preparation, with particular emphasis being placed on applications in pharmaceutical, environmental, food, clinical and bioanalytical fields.

[Click here to submit an abstract>>](#)

WHAT DO YOU GET?

Access to all conference and poster sessions
Access to the co-located vendor exhibition
Free entry to networking evening (27 July)
Tea/coffee, refreshments and lunches
Delegate pack including comprehensive conference handbook

REGISTRATION

Online registration for Separation Science Asia 2011 is now open and will be available until approximately one week prior to the conference start. All registrations made before 31 March, 2011 will apply for the Early Bird discount.

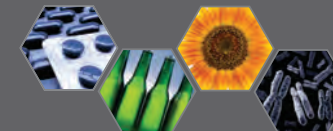
Also note, that during the registration process you will need to indicate if you would like to attend the networking social event on the evening of 27 July. This is free of charge to attend and part of your delegate fee.

[Click here for more information or to register>>](#)

[For programme enquiries>>](#)
david.hills@sepscience.com

[For sponsorship enquiries>>](#)
dean.graimes@sepscience.com

Supported by



www.sepscienceasia.com/conferences

Figure 4

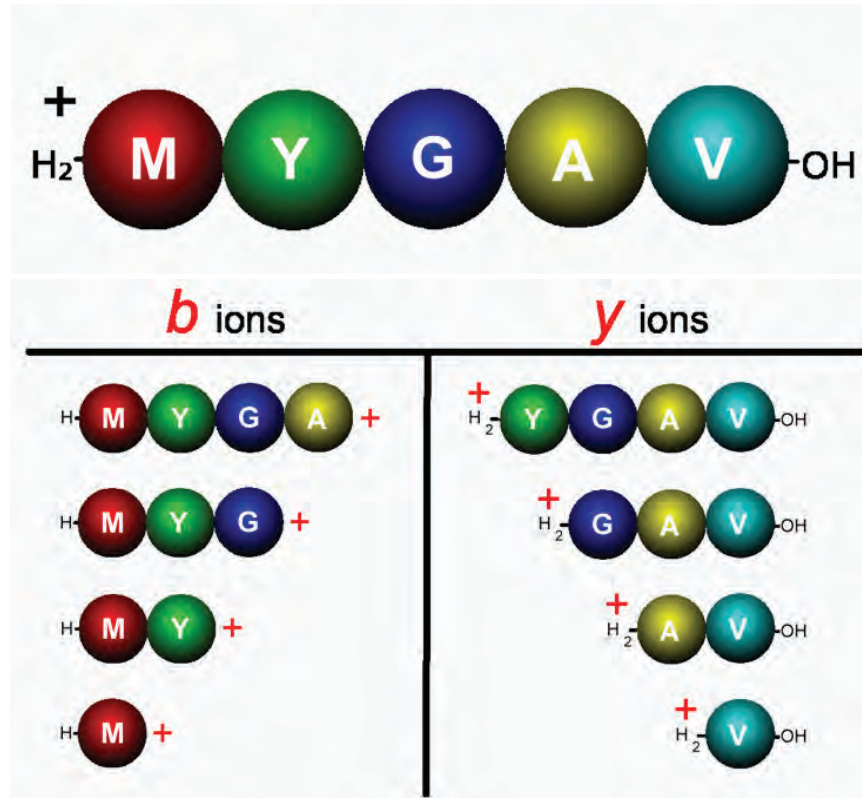


Figure 4: Penta-peptide single-charge precursor ion and the complete series of *b* and *y* product ions that may appear in an MS/MS spectrum.

reaction is a heterodimer as illustrated in the figure. There is some probability of the proton adducting to either the residue 3 or residue 4 amide nitrogen. Thus two fragment ions are probable, i.e., both a y_2 and a b_3 ion. The structure of these two ions is shown along with their corresponding neutral losses.

The proton mobilization theory also explains how CAD fragmentation of a single-charge peptide ion can result in formation of a complete or nearly complete series of ions of a particular

type. Figure 4 illustrates a penta-peptide single-charge precursor ion and the complete series of *b* and *y* product ions that may appear in an MS/MS spectrum. In Part 2 of this article we will show the spectrum resulting from this experiment and discuss in detail an interpretation strategy leading to a complete sequence determination.

Fred Klink is a trainer and consultant to the pharmaceutical, biotech, and chemical industries as well as law enforcement and other government laboratories. Fred's specialty is HPLC, LC/MS, and solid-phase extraction technologies.

Fred received a degree in biochemistry from Northwestern University and completed graduate studies and an internship in forensic chemistry at the University of Illinois. After graduation, Fred entered the analytical instruments industry where he spent seventeen years in varying positions from applications chemist, development project manager, and manager for strategic planning. Fred has been teaching highly regarded MS and LC/MS courses and providing consulting services since 1996.

Fred is the author of several journal articles and book chapters including the LC/MS entry in the *Wiley Encyclopedia of Analytical Chemistry*. He is a member of the American Chemical Society and American Society for Mass Spectrometry

Featured Applications



Comprehensive analysis of crude oil by two-dimensional GC (GCxGC) and time-of-flight (TOF) MS

Company: ALMSCO

Crude oil is the generic term for the unrefined flammable liquid that is mined from the ground. It contains vast amounts of organic compounds ranging from light hydrocarbons to complex biomolecules, derived from the remains of ancient marine organisms and bacteria. Within the complexity of crude oil, the compounds of most interest to the petroleum industry are relatively volatile (boiling points generally below 400°C) and non-polar, therefore separations are predominantly performed by GC with a non-polar column. The resulting chromatograms are highly convoluted and usually characterised by a matrix of unresolved material that appears as a significant background “hump” beneath the partially resolved non-polar compound peaks.

[Download](#)



Food Safety Analysis: LC/MS/MS Applications Using Core-Shell Technology HPLC Columns

Company: Phenomenex

The safety of our food supply has come under increasingly intense scrutiny with recent episodes of food products found tainted with melamine, antifreeze, salmonella, and potentially harmful antibiotics, to name a few of the higher profile examples. A newly developed, commercialized Kinetex 2.6 µm core-shell chromatographic particle offers the performance benefits of fully-porous sub-2 µm particles (increased chromatographic efficiency and resolution, shorter analysis times, and increased sensitivity) but at substantially lower operating pressures. The benefits provided by the core-shell technology are illustrated in three food safety LC/MS applications (antibiotics in meat, aflatoxins in peanut butter, and melamine and cyanuric acid in baby formula) on the three Kinetex phases currently available.

[Download](#)



Non-targeted Screening and Accurate Mass Confirmation of 510 Pesticides Using High Resolution Benchtop LC/MS

Company: Thermo Fisher Scientific

As agricultural trade grows and food safety concerns mount, stricter pesticide regulations are being enforced around the world. Increased pesticide testing and reductions in maximum permissible residue levels have driven demand for fast, sensitive and cost-effective analytical methods for high-throughput screening of multi-class pesticides in food. Detection of 510 pesticides at low ppb levels was achieved within 12 minutes using the Thermo Scientific Exactive benchtop LC/MS system powered by Orbitrap technology. The high resolving power of the Thermo Scientific Orbitrap platform enables accurate mass confirmation of all compounds, including isobaric pesticides. Accurate, robust, easy to use and cost-efficient, the Exactive LC/MS is ideally suited for routine, comprehensive screening of targeted and non-targeted pesticides at or below the 0.01 mg/kg (10 ppb) default limit set by EU and Japanese legislation.

[Download](#)

Recommend a Colleague

If you have a work colleague, collaborator or staff member who would benefit from this monthly publication then send us their details below.

[Recommend](#)

Read the latest **separation** | science
driving analytical chemistry forward

Accurate and Rapid Metabolomics Strategies Using GC-MS/MS-MRM Technology

