Strategic Trends and Developments in the Pharmaceutical/Biopharmaceutical Industry: Emerging Product Needs and Challenges

Mike S. Lee
Milestone Development Services

ALDA Breakfast Event
Pittsburgh Conference
March 8, 2016
Atlanta, Georgia
Global Pharmaceutical Industry Perspectives

- Research & Development Cost
  - No guarantee of success
- Market and Geographical Shift
  - Aging population
  - Prevention and diagnosis
  - Asia
  - Personalized medicine
- Diagnostics
  - Accurate and efficient
- Biotechnology
  - Genetic differences
  - Precise treatment

• On the Brink of a Healthcare Revolution

F.B. Humer, Hoffman-La Roche, 2005
Considerations in a Post-Genomic Society

- Clinical knowledge management
- Patient information
- Virtual patient
- **Personalized medicine**
- Disease at the atomic scale
  - DNA
  - Genetic vaccines
  - Biomemetic engineering
- Stem cells

- **The Desire for Longevity is a Key Driver**
  - Brain
  - Aging
Personalized Healthcare
Solutions for Healthcare

- Early detection of disease
  - Leads to earlier treatment
- Response-guided medication
  - Leads to better treatment
- Clinical trials to mirror patient therapy
  - Companion diagnostics
- Treatments tailored to specific sub-groups
  - Personalized medicine

• Future Treatments will be Tailored to Specific Subgroups
The Bioanalytical LC/MS/MS Flow Path

• The Endpoint is the Mass Spectrometer, Regardless of the Path! – Brad Ackermann, Eli Lilly


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Method Development Strategies

- Standard Methods
- Template Structure Identification
- Databases
- Screening
- Integration
- Miniaturization
- Parallel Processing
- Visualization
- Automation

- Transformation of the Mass Spectrometer into a Clinical Analyzer

M.S. Lee, *LC/MS Applications in Drug Development*, John Wiley & Sons; New York 2002
Attomole Protein Characterization by Capillary Electrophoresis–Mass Spectrometry

Gary A. Valaskovic, Neil L. Kelleher, Fred W. McLafferty*

Electrospray ionization with an ultralow flow rate (≤4 nanoliters per minute) was used to directly couple capillary electrophoresis with tandem mass spectrometry for the analysis and identification of biomolecules in mixtures. A Fourier transform mass spectrometer provided full spectra (>30 kilodaltons) at a resolving power of ≈60,000 for injections of $0.7 \times 10^{-18}$ to $3 \times 10^{-18}$ mole of 8- to 29-kilodalton proteins with errors of <1 dalton in molecular mass. Using a crude isolate from human blood, a value of 28,780.6 daltons (calculated, 28,780.4 daltons) was measured for carbonic anhydrase, representing 1 percent by weight of the protein in a single red blood cell. Dissociation of molecular ions from $9 \times 10^{-18}$ mole of carbonic anhydrase gave nine sequence-specific fragment ions, more data than required for unique retrieval of this enzyme from the protein database.

The molecular elucidation of biochemical events at the cellular and subcellular level is increasingly dependent on innovative methodologies for molecular characterization and imaging (1). These techniques have been especially successful for prese-

*To whom correspondence should be addressed.
Nanospray Ionization: 25 Years Later

• Diffusion Curve

Quantitative

Early Adopters 13.5%
Early Majority 34%
Late Majority 34%
Laggards 16%

Qualitative

2.5% Innovators

“Nanospray IS Electrospray”
- Rick King, 2006 CPSA

• Perceived Usefulness
  – Very small sample volumes
    • Limited sample available
  – Closer to a universal ionization
    • More compounds ionize
    • More compounds have a similar response

• Perceived Ease of Use
  – Difficult to setup initially
  – Difficult to keep running
  – Difficult to use with gradient LC

Courtesy of Dr. Rick King, PharmaCadence LLC
Integration and Miniaturization – The Future of High Performance LC/MS

Nathan Yates | University of Pittsburgh
Integrated and Miniaturized High Performance LC/MS – iPhone Analytics

• Integration and Miniaturization Improves Performance
Integration and Miniaturization – New Markets Demand Ease of Use!

• “Ease-of-Use is High Performance!”
  - Shane Needham, Alturas Analytics
Integration and Miniaturization – The Future of High Performance LC/MS

“The Column IS the Source.”
“Chromatography is analog, we need to digitize chromatography.”

Mike S. Lee
Milestone Development
(Circa 1998)

Bob Kennedy
University Michigan
Analysis of Samples Stored as Individual Plugs in a Capillary by Electrospray Ionization Mass Spectrometry

Jian Pei,† Qiang Li,† Mike S. Lee,‡ Gary A. Valaskovic,§ and Robert T. Kennedy*†

Departments of Chemistry and Pharmacology, University of Michigan, Ann Arbor, Michigan 48109-1055, Milestone Development Services, Milestone Development Services Inc., Newtown, Pennsylvania 18940, and New Objective, Inc., 2 Constitution Way, Woburn, Massachusetts 02139

(b) ESI plume
emitter tip → spraying

(c) Syringe pump

MS

High Voltage

Sample

Air

Oil

Rel. Abundance

(b) Relative Abundance

Time (sec)

0 18 36 54

50 25

no signal
Segmented Flow Nanospray

(A) 

Nano LC pump 
Capillary column 
Oil segmented separated components 

Oil Inlet 100 μm i.d. T junction HPFA+ tubing

(B) 

Syringe pump 
±1.5 kV 
LITQ MS

Coated tip 

(C) 500 μm

Anal. Chem. Vol. 82, No. 12, June 15, 2010 

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Going Digital: Format samples into a linear segmented array

- Well plate contains:
  - Unknown samples
  - Calibration standards
  - QC samples

- Re-format samples into a linear segmented array

- The segmented array can be used to:
  - Store samples
  - Process samples (prepare, derivatize, purify)
  - Deliver for analysis
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Blanks

Pump

ESI Nano Spray

Mass Spectrometer

Data Out
Going Digital: Format samples into a linear segmented array

A linear array capable of:
- long term storage
- easy handling
- automation compatible
- bio-inert flow path

Wide sample volume: Picoliter to microliter

US Patent: 8,431,888
What can you do with “Infinite” Speed?

Collect an Individual QA Curve for Each Sample

Lucinda Cohen | Merck

Samples for Analysis (triplicate)  QA “Curve” (triplicate)
Combining Segmented Flow with DBS

FEP Sampling from 5uL of extraction solvent (50% MeOH) on top of DBS.

0.2 uL samples with 0.2 uL air-gaps. Larger spacing between different concentrations from card.
Segmented Flow Enables Fast Chemical Processing

Courtesy of Robert Kennedy, University of Michigan
Segmented Array MS is (very) Fast

10 individual samples

Product (m/z 465.5)

Substrate (m/z 622.5)

23 sec.

Time (3.4 min)

90 Samples from an enzyme inhibition assay analyzed in 3.4 min.

Courtesy of Robert Kennedy, University of Michigan
Analog-to-Digital Chromatography (ADC)
Stored chromatogram is then analyzed off-line on the MS
Segmented Flow Preserves the Quality of the LC (no diffusion)

- Acquire 3 LC-MS traces in time for 1
- Improve throughput (multiple LC for 1 MS)

Off-line RIC at 3x flow rate

On-line nanoLC-MS

Courtesy of Robert Kennedy, University of Michigan
Segmented array can store samples/separations over a long period

Gradient concentration of segments stored April 2013

Taylor Dispersion is controlled.
“I submit that a metabolic profile is a graphical representation of an individual's metabolic status as it forms a picture/outline of their metabolism.” –
CPSA Metabolomics 2015

Associations between Phenylalanine-to-Tyrosine Ratios and Performance on Tests of Neuropsychological Function in Adolescents Treated Early and Continuously for Phenylketonuria

Monica Luciana, Jill Sullivan, and Charles A. Nelson
Mass Spectrometry and Chromatography for Healthcare

- Early detection
  - Mass Spectrometer Transformed into a Clinical Analyzer
  - A Molecular Microscope
- Response-guided medication
  - Chromatography-Mass Spectrometry Platforms Evolve into a Clinical Analyzer
  - A Molecular Plate Reader

- Mass Spectrometry and Chromatography Uniquely Positioned to Enable Personalized Healthcare
  - Integration, Miniaturization and Visualization are Critical for Success