The Need for Speed: Is your LC or Mass Spectrometer the Top Gun for Improving Throughput?

LCMS & Related Topics Interest Group
ASMS June 2017

Russel Grant
Labcorp

Erin Baker
PNNL

Scott Mellors
908 Devices

Erik Soderblom

Will Thompson

Matt Foster and Tricia Ho

Panelists

Scott Mellors, PhD

Senior Research Scientist

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Erin Baker, PhD

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Pacific Northwest
NATIONAL LABORATORY

Russ Grant, PhD

VP Research and Development

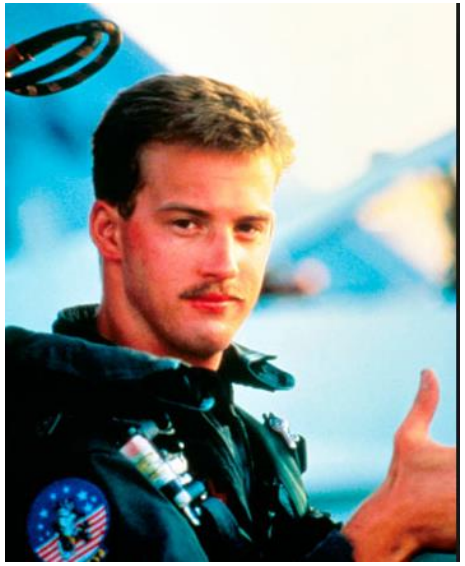
LabCorp
Laboratory Corporation of America

Panelists

Scott Mellors, PhD

Senior Research Scientist

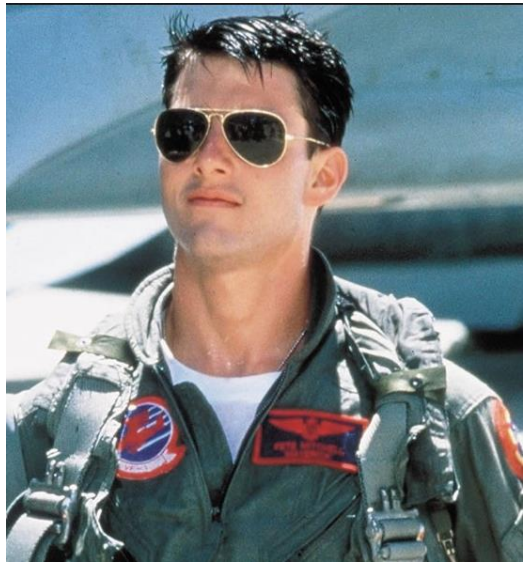
“Goose”



Erin Baker, PhD

Senior Research Scientist

“Maverick”



Russ Grant, PhD

VP Research and Development

“Iceman”

Workshop Survey

- Instrument type?
- Classes of analytes?
  - Peptides
  - Proteins/Antibodies
  - Metabolites/Small Molecules
  - Drug Metabolism/Pharmacokinetics
  - Environmental
  - Petroleum/Fuel
- Current Bottleneck in Analytical workflow?
  - Sample Prep? Separations? Mass Spectrometry?

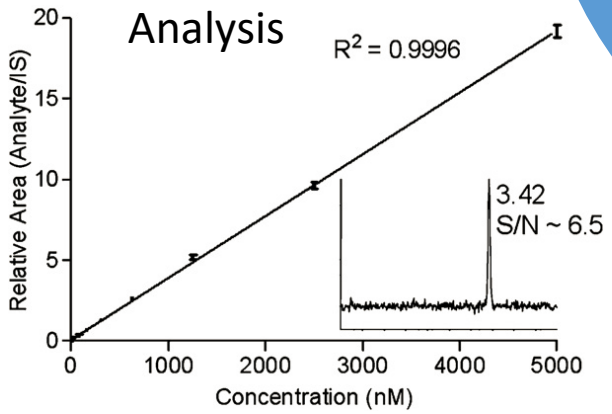
The Big Picture

Sample Collection, Storage, Organization

Sample Preparation

Analyte Separation

MS Acquisition



Data Analysis

Data Reporting

The Big Picture

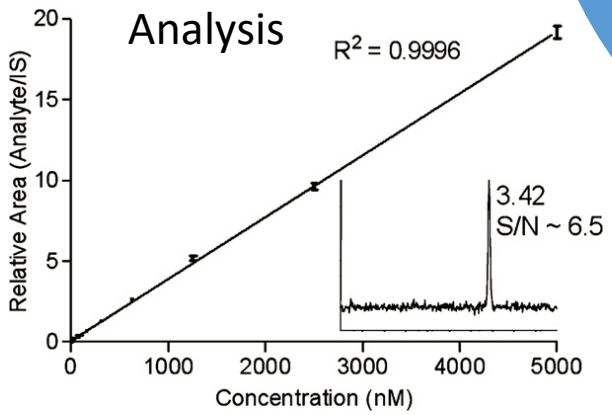
Sample Collection, Storage, Organization

Sample Preparation

Analyte Separation

MS Acquisition

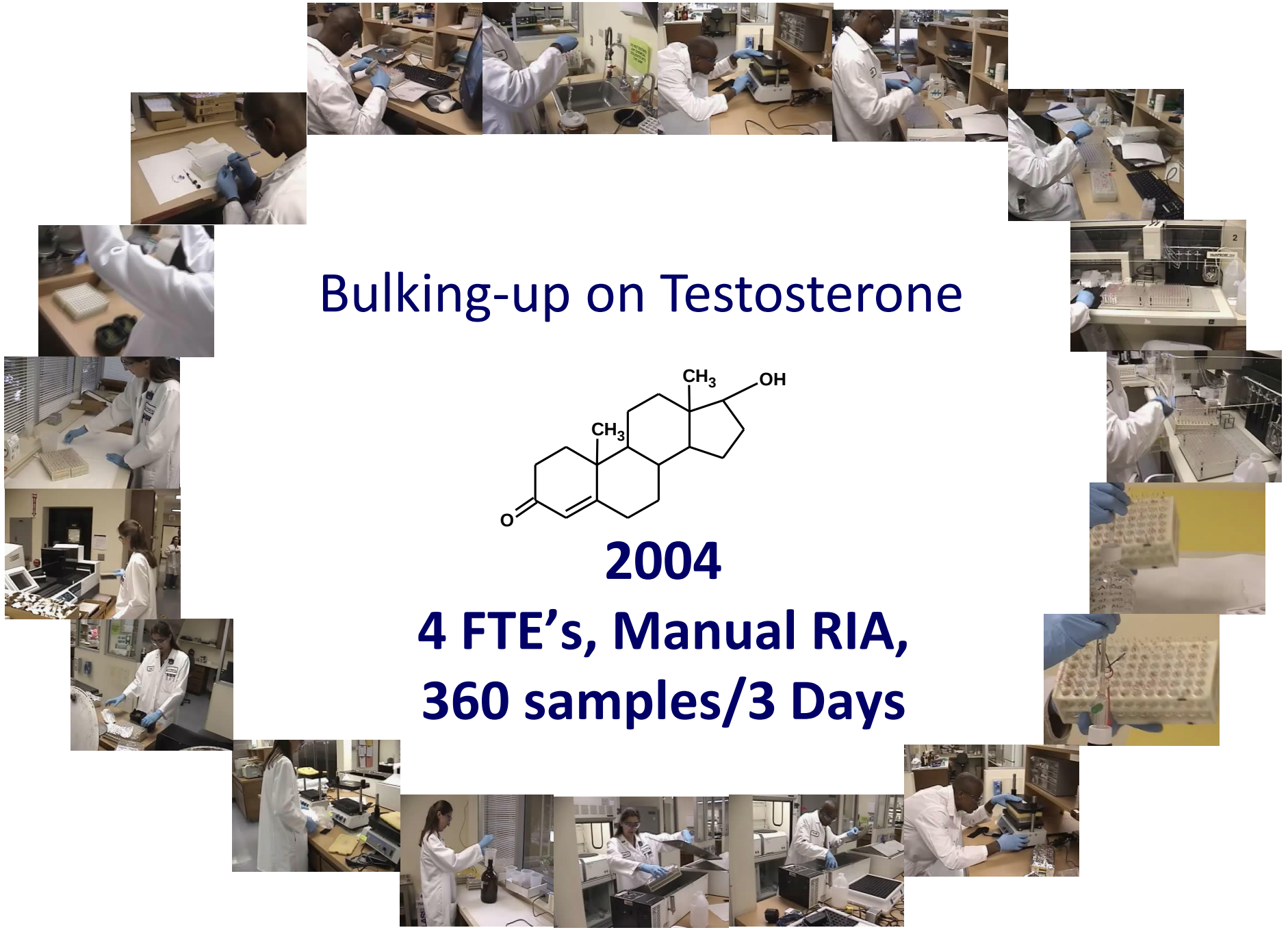
Data Analysis



Data Reporting

Russell Grant, Ph.D.

Laboratory Corporation of America



Bulking-up on Testosterone

2004

4 FTE's, Manual RIA,

360 samples/3 Days

Why Change?

Clinical Chemistry 49:8
1381–1395 (2003)

Endocrinology and Metabolism

Testosterone Measured by 10 Immunoassays and by Isotope-Dilution Gas Chromatography–Mass Spectrometry in Sera from 116 Men, Women, and Children

Joëlle Taieb,[1] Bruno Mathian,[2] Françoise Millot,[3] Marie-Claude Patricot,[2] Elisabeth Mathieu,[4] Nicole Queyrel,[5] Isabelle Lacroix,[6] Claude Somma-Delpero,[7] and Philippe Boudou[8*]

Background: **Commercially available testosterone immunoassays give divergent results, especially at the low concentrations seen in women. We compared immunoassays and a nonimmunochemical method that could quantify low testosterone concentrations.**
Methods: **We measured serum testosterone in 50 men, 55 women, and 11 children with use of eight nonisotopic immunoassays, two isotopic immunoassays, and isotope-dilution gas chromatography–mass spectrometry (ID/GC-MS).**
Results: **Compared with ID/GC-MS, 7 of the 10 immunoassays tested overestimated testosterone concentrations in samples from women; mean immunoassay results were 46% above those obtained by ID/GC-MS. The immunoassays underestimated testosterone concentrations in samples from men, giving mean results 12% below those obtained by ID/GC-MS. In women, at concentrations of 0.6–7.2 nmol/L, 3 of the 10 immunoassays gave positive mean differences >2.0 nmol/L (range, −0.7 to 3.3 nmol/L) compared with ID/GC-MS; in men at concentrations of 8.2–58 nmol/L, 3 of the 10 immunoassays tested gave mean differences >4.0 nmol/L (range, −4.8 to 2.6 nmol/L).**
Conclusion: **None of the immunoassays tested was sufficiently reliable for the investigation of sera from children and women, in whom very low (0.17 nmol/L) and low (<1.7 nmol/L) testosterone concentrations are expected.**

The measurement of circulating testosterone is clinically relevant in the investigation of androgen disorders in humans (*1*). In men, testosterone analysis is used to evaluate the endocrine activity of the testis. In association with gonadotropin determination, the circulating testosterone concentration provides information concerning the origin of testicular dysfunction (*2*, *3*). Measurement of testosterone is also recommended in the monitoring of patients with metastatic prostate cancer treated with gonadotropin-releasing hormone analogs and/or by antiandrogen therapy, and as a means of checking the that testosterone concentration has decreased to the castration range (*4*–*7*). In women, testosterone is frequently measured as part of the investigation of alopecia, acne, and/or hirsutism (*8*, *9*), although testosterone concentrations remain within the reference interval in 50% of cases (*10*). Simple testosterone measurements have been shown to have predictive value for the dissection of androgen-secreting tumors of ovarian origin (*11*) and have also been used to define the minimum drug dose required to abolish androgen secretion in hyperandrogenic women (*12*). This steroid has therefore been closely monitored in the follow-up of patients with congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency (*13*, *14*).

In children, the circulating testosterone concentration is determined principally for the diagnosis, treatment,

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[4] Biochemistry and Molecular Biology Laboratory, Angers Hospital, 49033 Angers, France.
[5] Biochemistry Laboratory, Versailles Hospital, 78150 Le Chesnay, France.
[6] CERBA Laboratory, 95066 Cergy Pontoise, France.
[7] Nuclear Medicine Laboratory, Timone Hospital, 13385 Marseille, France.
[8] Unit of Hormonal Biochemistry, St Louis Hospital, 75010 Paris, France.
* Address correspondence to this author at: Unit of Hormonal Biochemistry, St Louis Hospital, Assistance-Publique-Hôpitaux de Paris, 1 avenue Claude Vellefaux 75010 Paris, France. Fax 33-1-42-49-42-80; e-mail philippe.boudou@bla.ap-hop-paris.fr.
Received March 5, 2003; accepted May 9, 2003.

Immunoassays for Testosterone in Women: Better than a Guess?

Recent developments in the field of mass spectrometry have provided the accuracy and sensitivity to evaluate very-low-abundance steroids such as testosterone in female and pediatric patients. In this issue of *Clinical Chemistry*, Taieb et al. (*1*) present the most comprehensive evaluation of automated testosterone immunoassays to date. They compared 10 commercially available immunoassays with isotope-dilution gas chromatography–mass spectrometry (ID-GC/MS) and reached the inescapable conclusion that testosterone immunoassay results for specimens from females are inaccurate. Similar data have been reported for individual testosterone immunoassays previously (*2*), but Taieb et al. (*1*) are the first to show that for every commercially available testosterone assay studied, the values are in error—by a factor of 2 on average and in some cases by a factor of almost 5. Are assays that miss target values by 200–500% meaningful? Guessing would be more accurate and additionally could provide cheaper and faster testosterone results for females—without even having to draw the patient's blood.

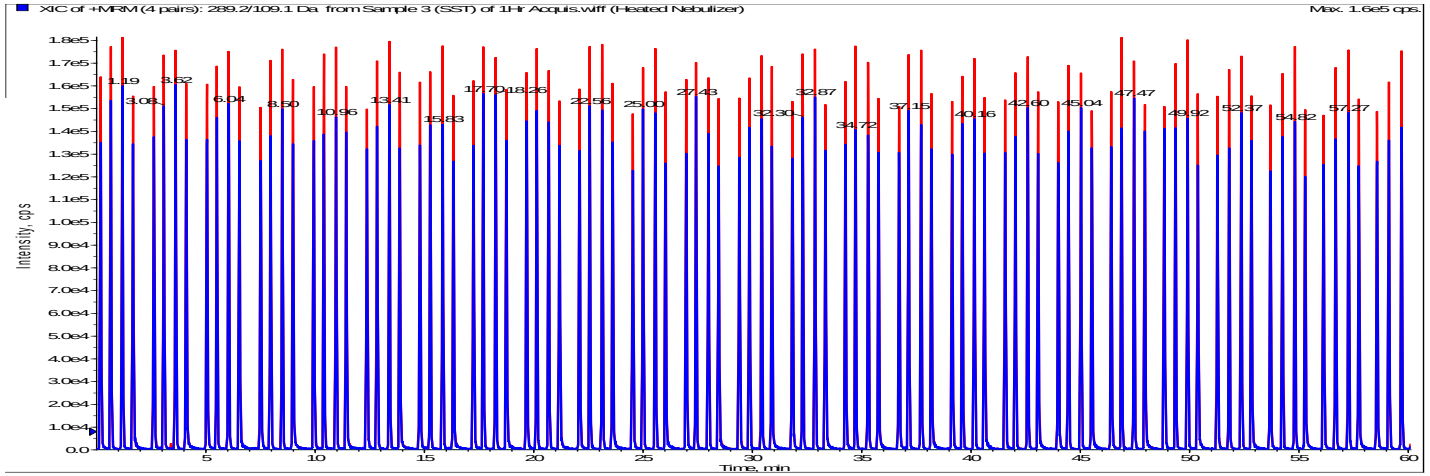
By limiting all guesses to a narrow range, e.g., 2.04–2.44 nmol/L, the results would rarely be off by more than a factor of 3. Using a random number generator, we generated values close to the average female concentration measured by Taieb et al. (they were kind enough to share their data with us as an aid to writing this editorial). A Bland–Altman plot for guessed values vs ID-GC/MS values had a mean difference for the 55 female samples of 0 nmol/L with a SD of the differences of 1.2 nmol/L. This SD compares favorably with those presented by Taieb et al. (*1*) in Table 4. Although not intended to be a statistically rigorous proof that random numbers are better than measuring female testosterone values with immunoassays, guessing appears to be nearly as good as most commercially available immunoassays and clearly superior to some!

Because medical test decisions are not made in a vacuum, a patient's appearance and presenting complaints would give the person guessing the serum testosterone concentration important information. Women with rapidly evolving signs and symptoms of virilization will have dramatically increased testosterone [>10.4 nmol/L (300 ng/dL)] (*3*), whereas women with late-onset 21-hydroxylase deficiency have moderately increased testosterone [~4.2 nmol/L (~120 ng/dL)] (*4*). Using this information while making an educated guess should give dramatically improved results. This would make educated guessing the better choice with the added benefits of rapid turnaround time and very low cost.

What are the implications of the results of the study by Taieb et al. (*1*) for epidemiologic research? A recent study by Dorgan et al. (*5*) designed to address this issue concluded "that although absolute concentrations may differ for some hormones, RIA and mass spectrometry can yield similar estimates of between subject differences in serum concentrations of most steroid sex hormones commonly measured in population studies". The testosterone assay that Dorgan et al. were comparing with MS included an extraction and column purification. Many people believe that liquid-liquid extraction combined with column purification before RIA analysis provides accurate results for testosterone in specimens from females. However, we have previously demonstrated that RIAs that include extraction and column purification steps do not agree well with ID-GC/MS (*6*). An important limitation in the study by Dorgan et al. (*5*) is that for female specimens they tested only sample pools (low, mid, and high). Determining how the assay would work on individual patient samples is not possible when pooled samples are used. This is a critical flaw, because clinicians are concerned about the concentration of testosterone in an individual; in contrast, when pooled samples are analyzed, any cross-reacting substances in an individual sample are diluted in the rest of the pool. In Fig. 1 of their report, Taieb et al. (*1*) show that there is a wide degree of scatter when an extraction chromatography RIA is compared with ID-GC/MS for individual specimens. Although it does appear that extraction chromatography RIA is slightly more accurate than commercially available testosterone immunoassays, until an extraction chromatography RIA has been properly validated, results from epidemiologic studies based on these methodologies are also suspect.

How can assays that are grossly inaccurate gain approval for use in diagnosis and treatment of endocrine abnormalities? Several factors warrant consideration. In the US, the Food and Drug Administration approval process for a new diagnostic assay when there is an existing, approved diagnostic assay consists of demonstrating substantial equivalence to a predicate assay in a premarket notification 510(k) process. For testosterone, one of the predicate devices that is acceptable for demonstrating substantial equivalence is the Chiron ACS-180 testosterone assay. Several years ago, we compared the ACS-180 testosterone assay with ID-MS. The ACS-180 did not provide reliable results for female specimens (*2*). If the predicate device is not accurate, how can the newly designed assay hope to function properly in a clinical setting? This feature of the 510(k) process is one reason that our profession has made little progress in developing clinically acceptable testosterone immunoassays. From our clinical laboratory perspective, we suggest that predicate devices need to be validated by an independent chemical technique, preferable by a reference (or definitive) method (*7*, *8*), before they are accepted as the standard to establish substantial equivalence. With the current regulatory environment, clinical chemistry is allowed, or perhaps even legislated, to perpetuate substandard levels of performance.

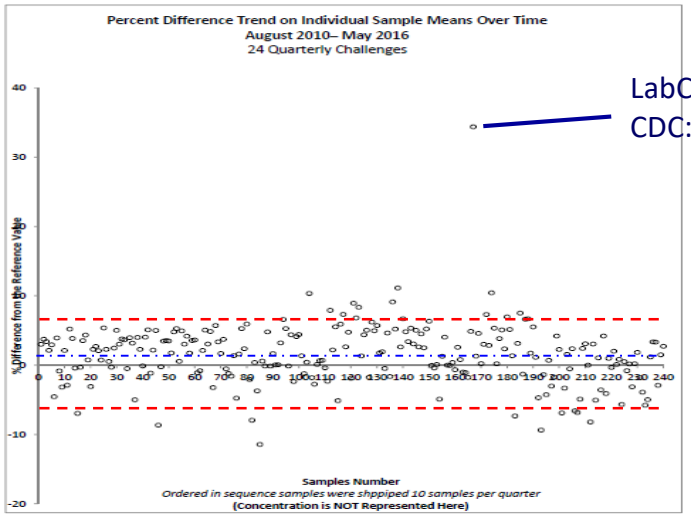
Recently, attention has focused on the need for better reporting of diagnostic accuracy of laboratory tests in peer-reviewed journals (*9*). Clearly, diagnostic accuracy is



99 samples in 60 minutes *or 2376 samples/system/day – Cycle time = 2.1 min*

2017 – 6 FTE's, 4800 samples/day/<24 hours

7 years CDC Certification (1120 samples)



LabCorp: 73 pg/mL
CDC: 56 pg/mL

Mean Bias = ~2%
Budget = ± 6.4%

Table 3. Percent of specimens that met desirable and minimal performance criteria from currently certified assays in CDC HoSt program.[a]

Parameter and performance criteria	Assay A	Assay B	Assay C	Assay D	Assay E
Imprecision,[b] n = 40					
Desirable (5.3%)	93 (100/85)	68 (80/55)	97 (100/93)	55 (75/35)	70 (75/65)
Minimal (8.0%)	100 (100/100)	85 (100/70)	100 (100/100)	85 (100/70)	80 (80/80)
Bias,[c] n = 40					
Desirable (6.4%)	98 (100/95)	48 (65/30)	54 (80/20)	55 (65/45)	88 (90/85)
Minimal (9.5%)	100 (100/100)	65 (95/35)	66 (95/27)	80 (95/65)	100 (100/100)
TE,[d] n = 160					
Desirable (16.7%)	100 (100/100)	79 (99/59)	67 (95/30)	89 (96/83)	98 (99/96)
Minimal (25.1%)	100 (100/100)	93 (100/86)	76 (100/45)	98 (100/96)	100 (100/100)

[a] Each assay was challenged with 40 specimens that had been assigned testosterone concentration based on the CDC reference method for quantifying testosterone. The 40 specimens were analyzed by each assay 4 different times over the course of 1 year (n = 160). Data are % all (% male/% female). Assay A, LabCorp, LC/MS/MS; Assay B, Boston University Steroid Hormone Assay Laboratory Section of Endocrinology, Diabetes, and Nutrition, Boston University School of Medicine, and Boston Medical Center, LC/MS/MS; Assay C, Roche Diagnostics, electrochemiluminescence; Assay D, Mayo Clinic, LC/MS/MS; Assay E, Covance Central Laboratories Services, LC/MS/MS.
[b] CV of the 4 individual measurement of each specimen.
[c] Percent difference between the mean of 4 replicate measurements and the assigned value.
[d] Percent difference of an individual measurement (n = 160) so that the specimens' imprecision is combined with evaluation of bias for each individual measurement.

Bothello et al, Clin Chem. 2013

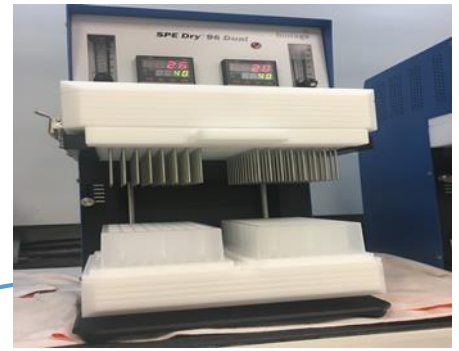
Positive Pressure Manifold

120 to 30 min
Batch Build/LIMS

SLE

200 – 45 min
Bi-directional
Heating

8/96 tip Automation



Evaporation

How and Why?

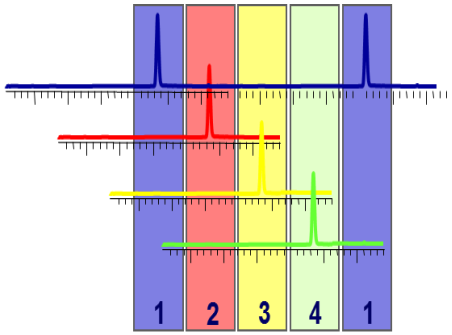
Automated “Cloud Based”
Data reduction

200% - 10% Data review
5 min post acquisition

70 – 38 sec

DLWII

24s window selected
from 2.1 min LC time
4 runs in parallel



Staggered Parallel LC

1100 – 1260 pump
Fused Core LC
4000 – 5500 MS

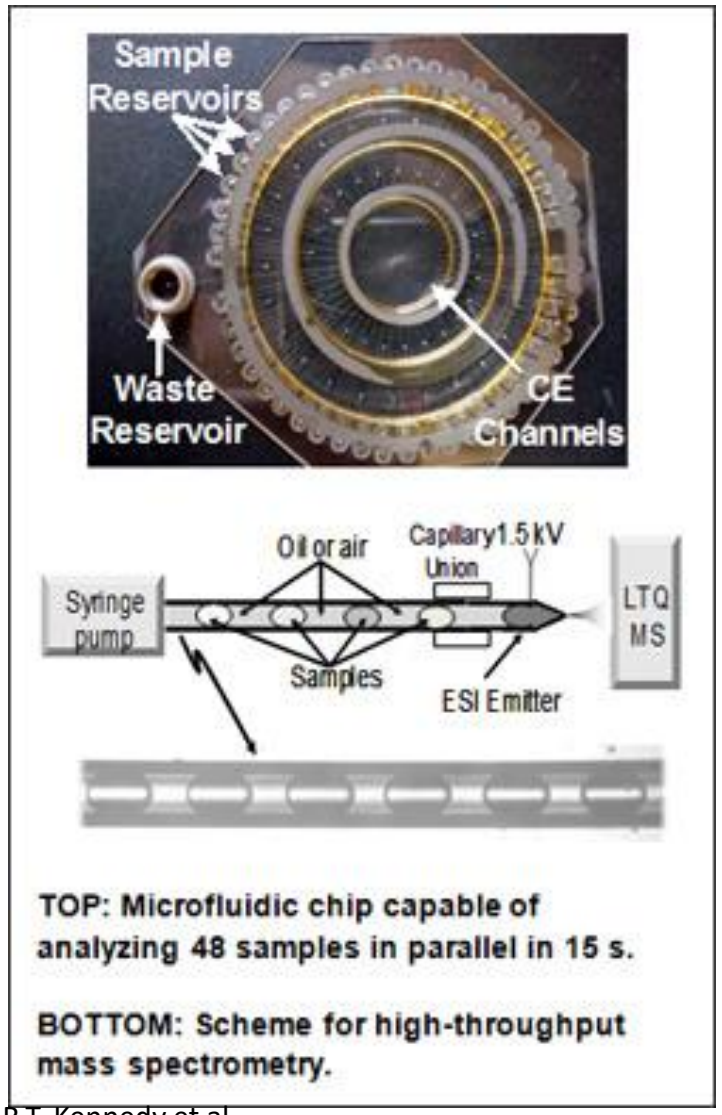
ARIA 4-Channel LC
Multiplexing - API5500

Scott Mellors, Ph.D.

908 Devices, Inc.

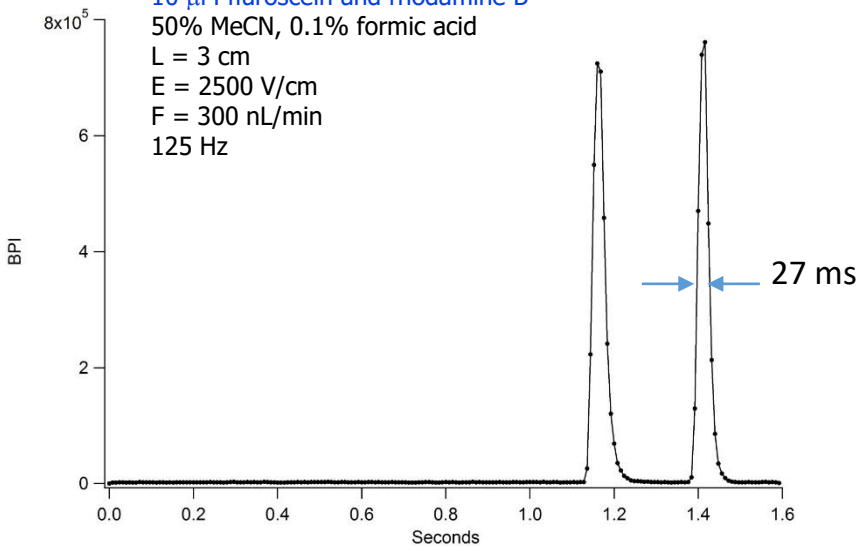
Can microfluidics contribute to high throughput “LC-MS” analysis?

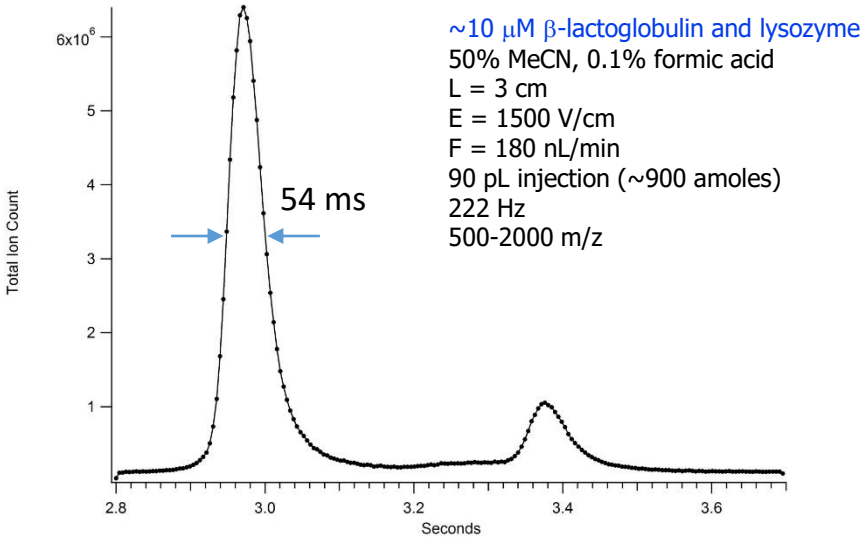
Kennedy Lab Work



R.T. Kennedy et al.
http://kennedygroup.lsa.umich.edu/research/high-throughput-analysis/

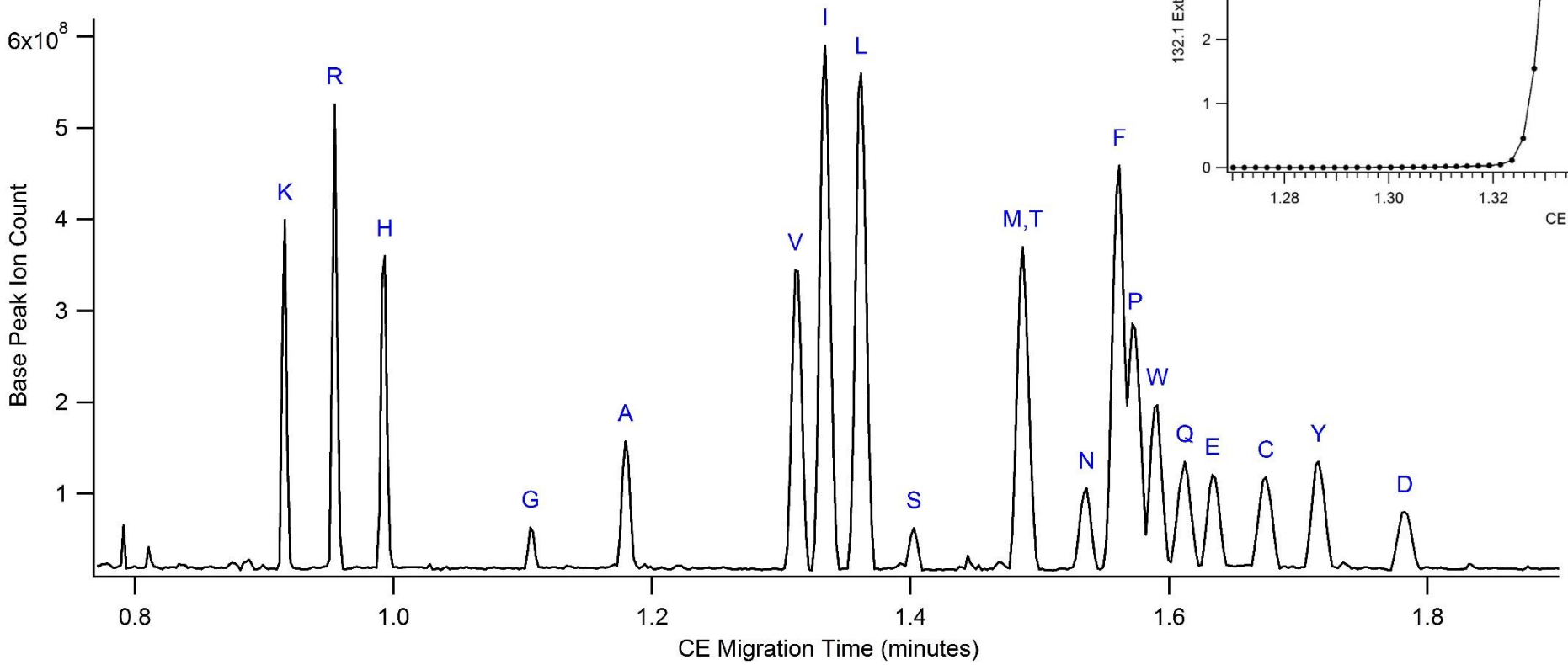
ZipChip™ CE-ESI-MS

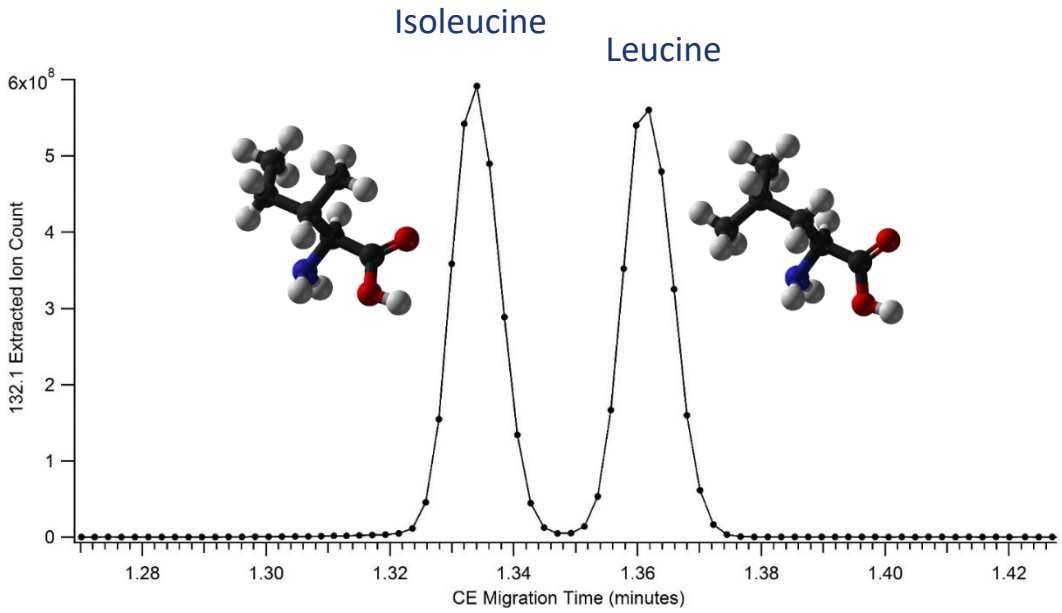




ZipChip Separation of Amino Acids

2.5 μM Promega Complete AA Mix
ZipChip HS (L = 10 cm)
ZipChip Metabolite Kit
Exactive EMR (R = 17,500)
Field Strength: 1000 V/cm
8.3 Hz



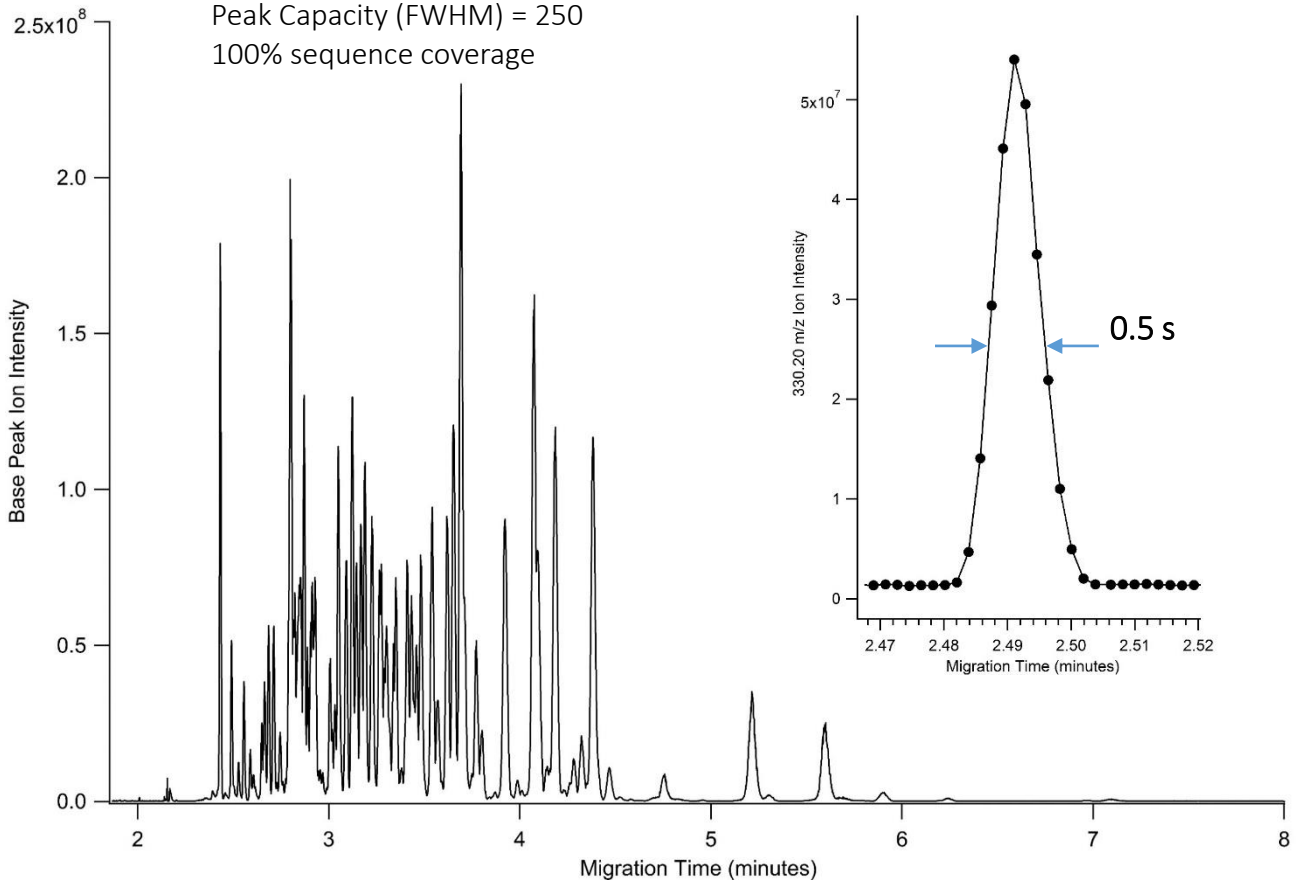


ZipChip Separation of Intact Proteins and Peptides

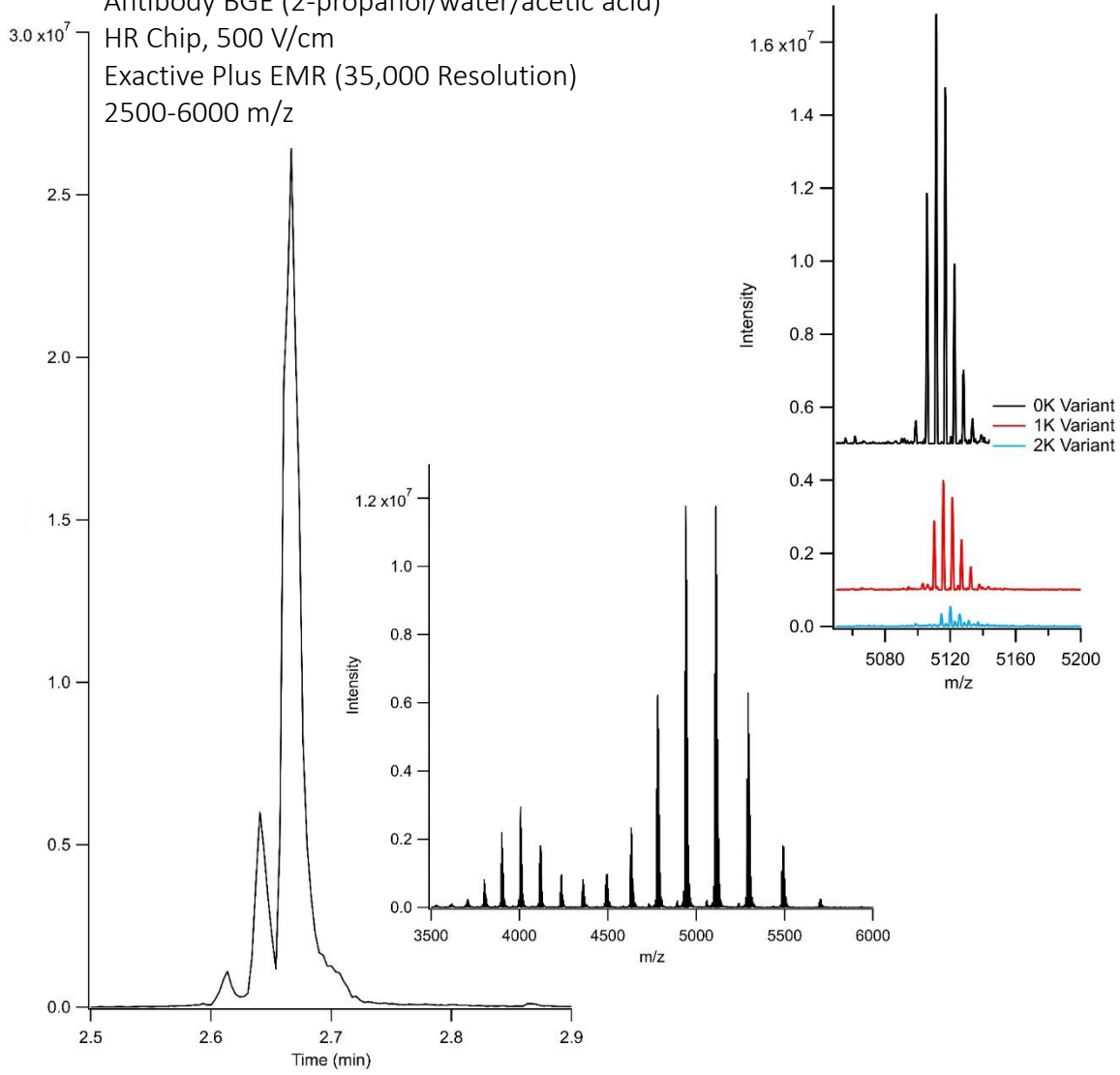
β galactosidase tryptic digest
Diluted in peptide kit diluent (5 fmoles injected)
Peptide BGE (acetonitrile/water/formic acid)
HR Chip, 500 V/cm
QExactive HF (30,000 Resolution)
300-2000 m/z

Peak Capacity (FWHM) = 250
100% sequence coverage

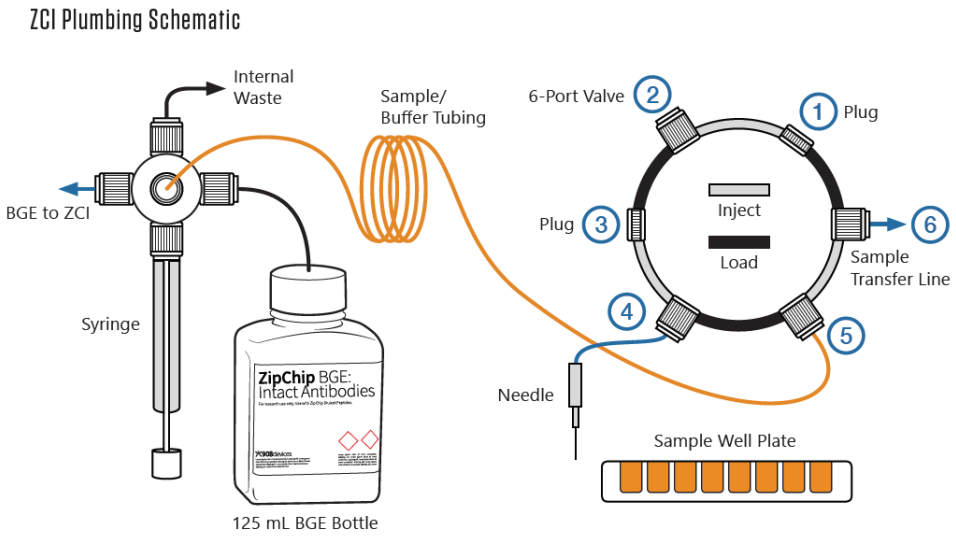
10 Hz at 30,000 Resolution



NIST monoclonal antibody reference material
Diluted in water to 0.5 mg/mL (0.25 pg injected)
Antibody BGE (2-propanol/water/acetic acid)
HR Chip, 500 V/cm
Exactive Plus EMR (35,000 Resolution)
2500-6000 m/z



Automation of Microfluidic CE-MS

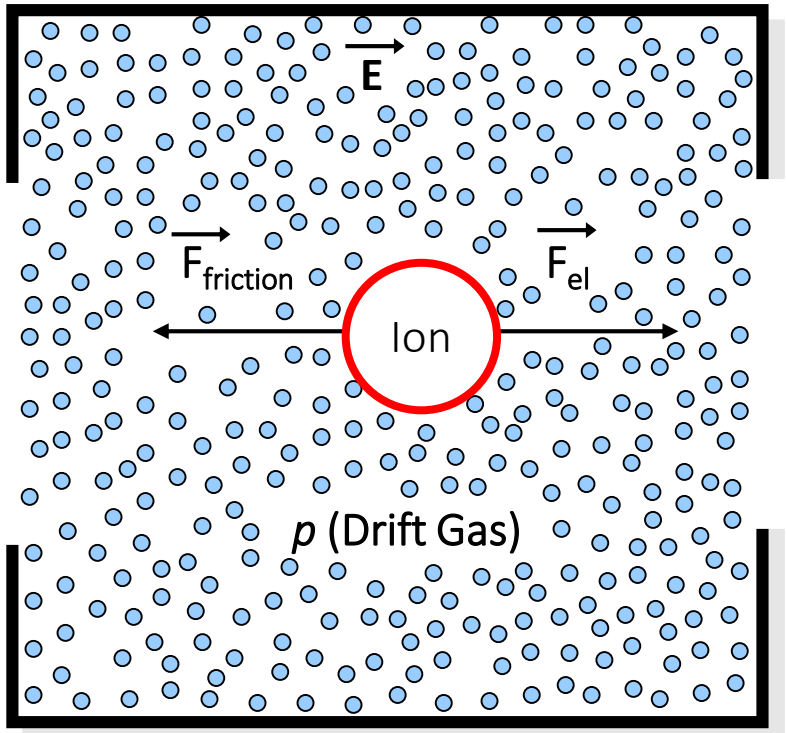


	Metabolites	Peptide Mapping	Intact Antibodies
Chip type	HS	HR	HR
CE run time	2-5 minutes	8 minutes	3 minutes
Peak capacity (FWHM)	~200	~250	n/a
Migration time reproducibility	2% CV	2% CV	1% CV
Peak area reproducibility	15% CV	15% CV	11% CV
Carryover	0.10%	0.10%	0.20%
Linear dynamic range	0.001 - 100 μM	0.001 - 100 μM	0.001 - 1 mg/mL
Limit of detection	1-10 nM	1-10 nM	0.001 mg/mL
Total cycle time	5-8 minutes	11 minutes	6 minutes
Time to run 100 samples	9-14 hours	19 hours	10 hours

Erin Baker, Ph.D.

Pacific Northwest National Laboratory

Ion mobility concept



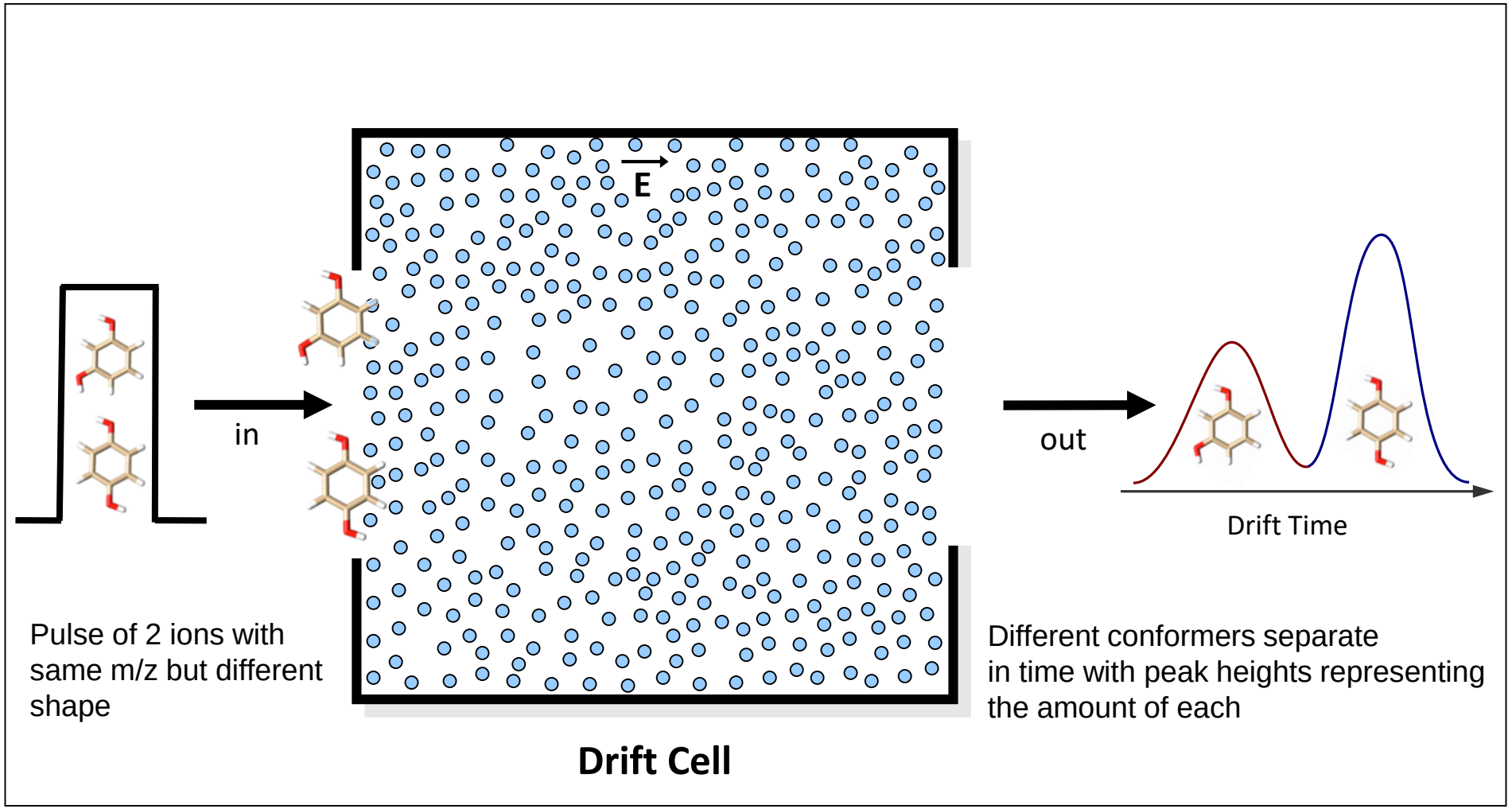
Drift Cell

velocity is constant

$$v = K \vec{E}$$

K = ion mobility

Ion mobility concept

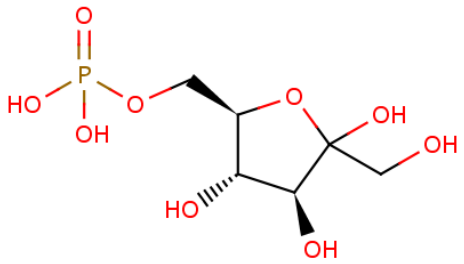


velocity is constant

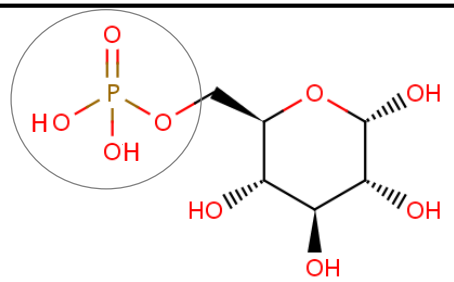
$$v = K\vec{E}$$

K = ion mobility

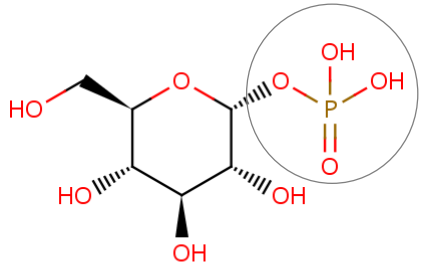
Isomers difficult to separate with hydrophobic interaction liquid chromatography (HILIC)



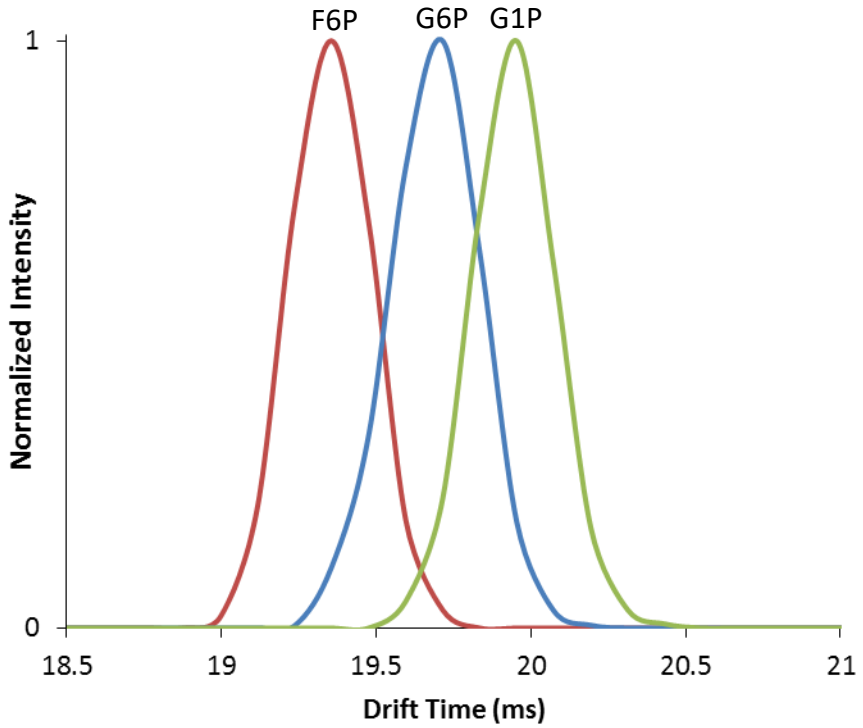
D-Fructose-6-phosphate (F6P)

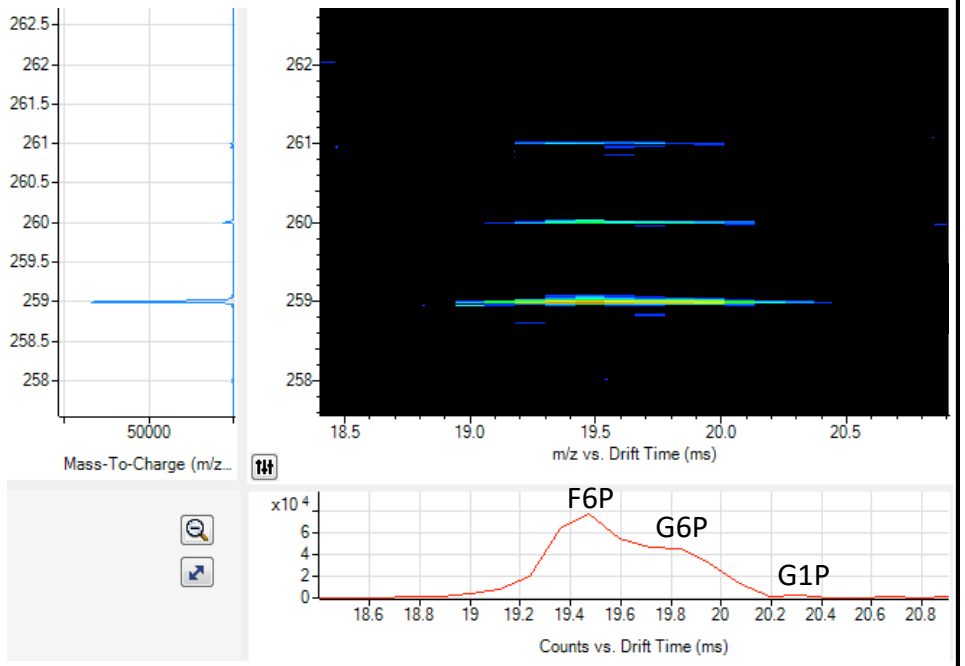


D-Glucose-6-phosphate (G6P)



α-D-Glucose-1-phosphate (G1P)

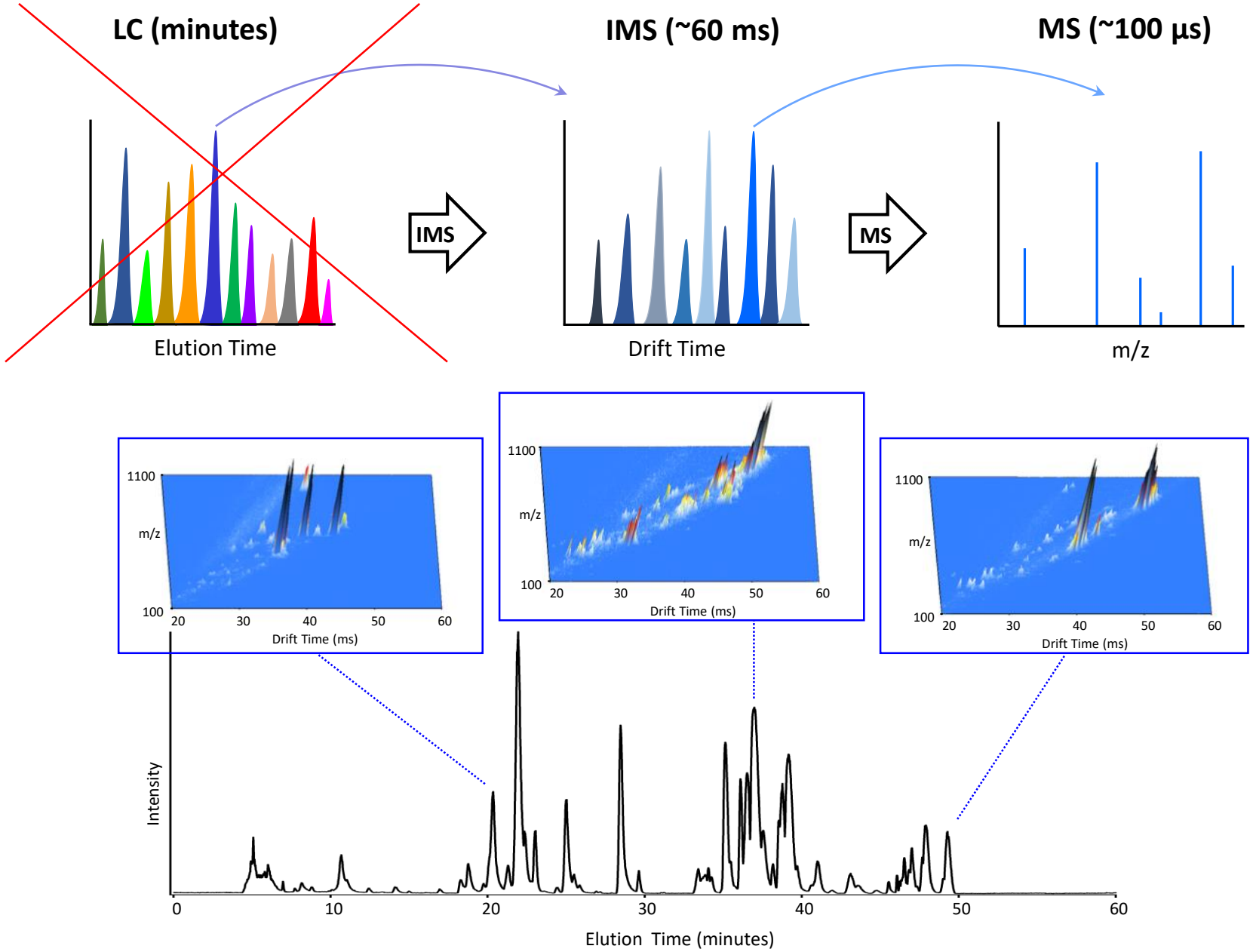




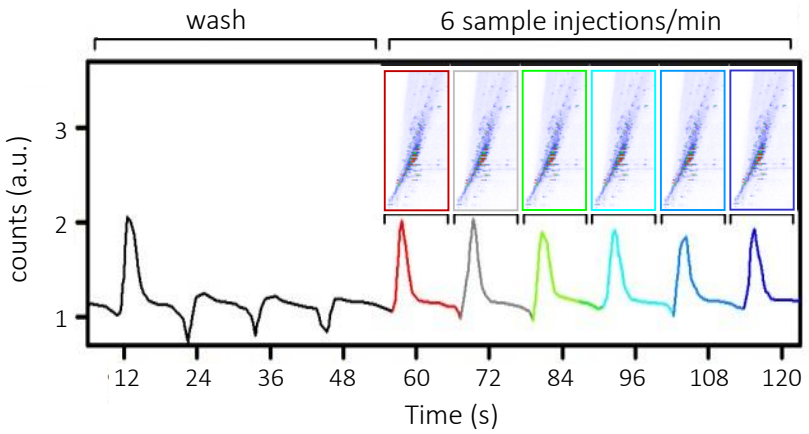
Deprotonated form $[M - H]^-$ m/z = 259.02

X. Zhang, et al. Clinical Mass Spectrom. 2016.

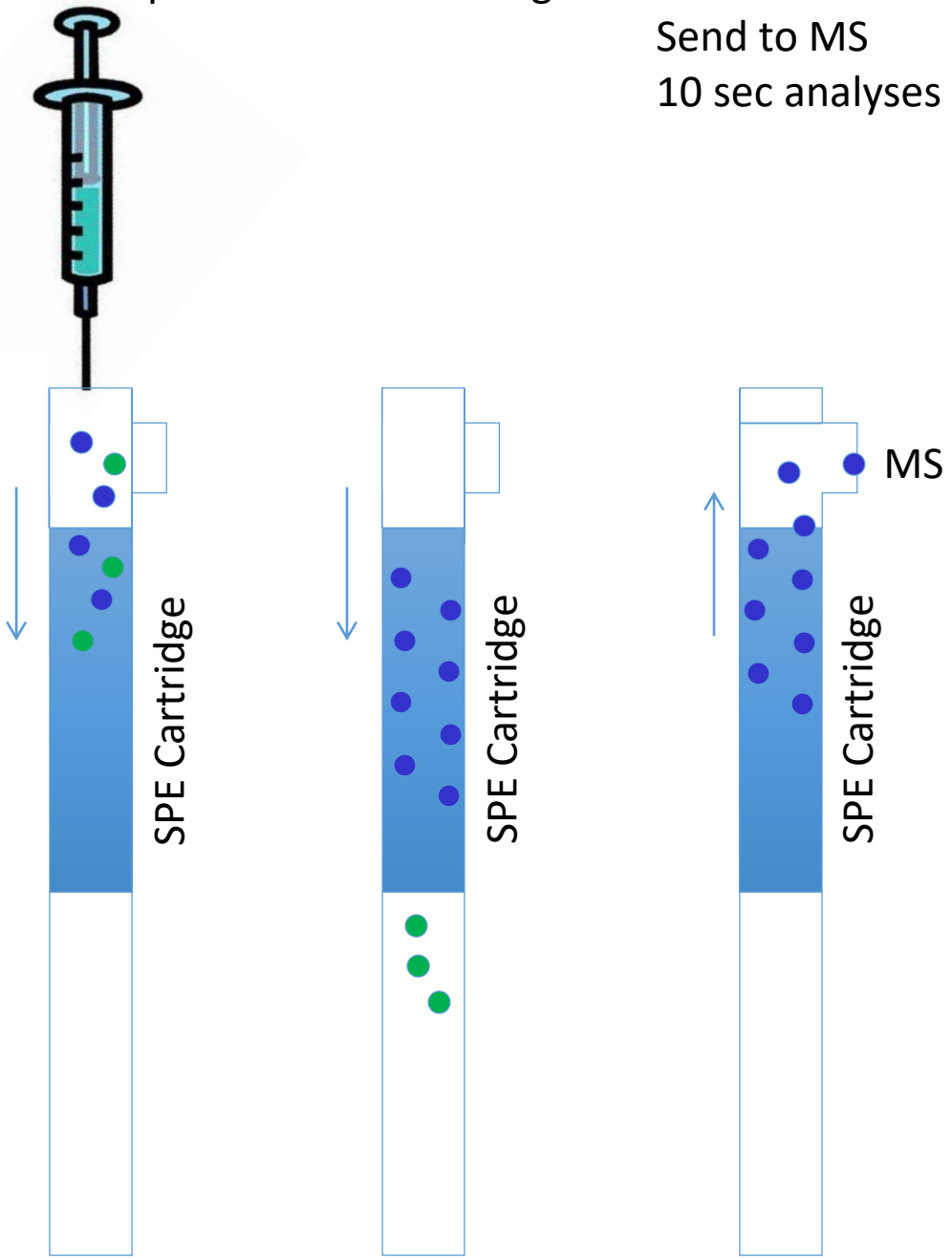
Ion mobility concept



Automated SPE system



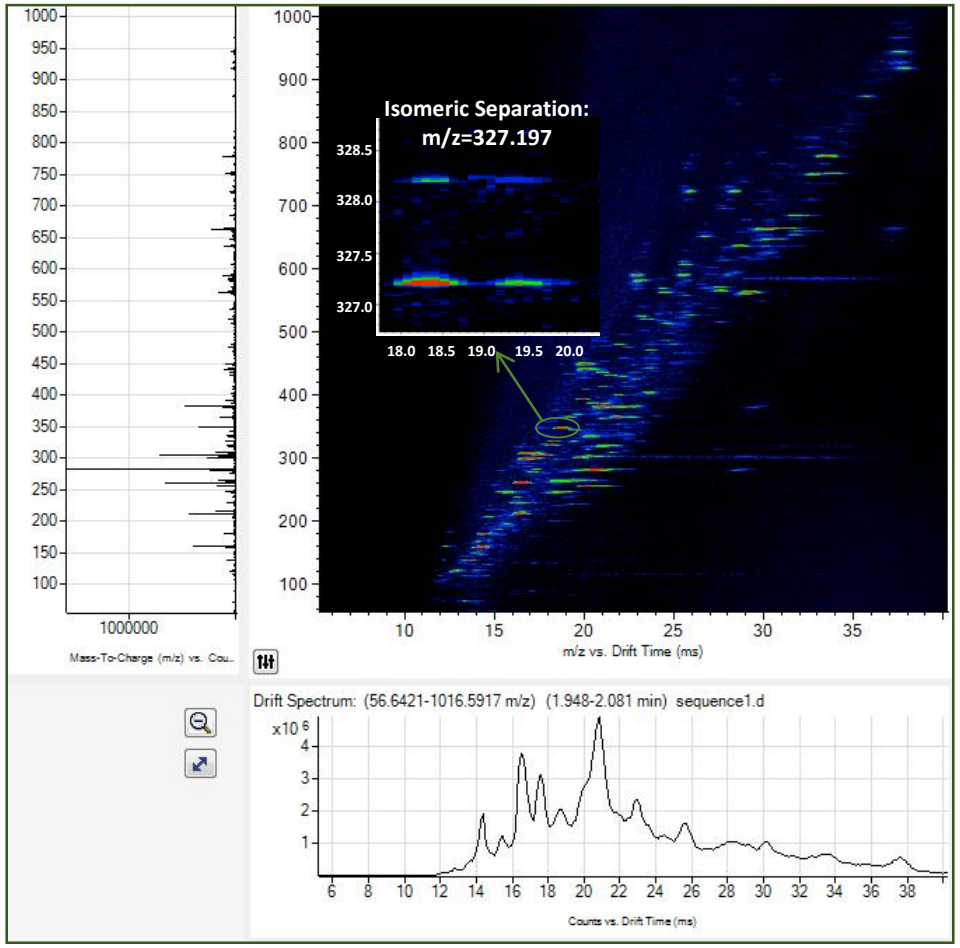
1. Inject Sample
2. Wash Cartridge
3. Reverse Flow
Send to MS
10 sec analyses



X. Zhang, et al. Clinical Mass Spectrom. 2016.

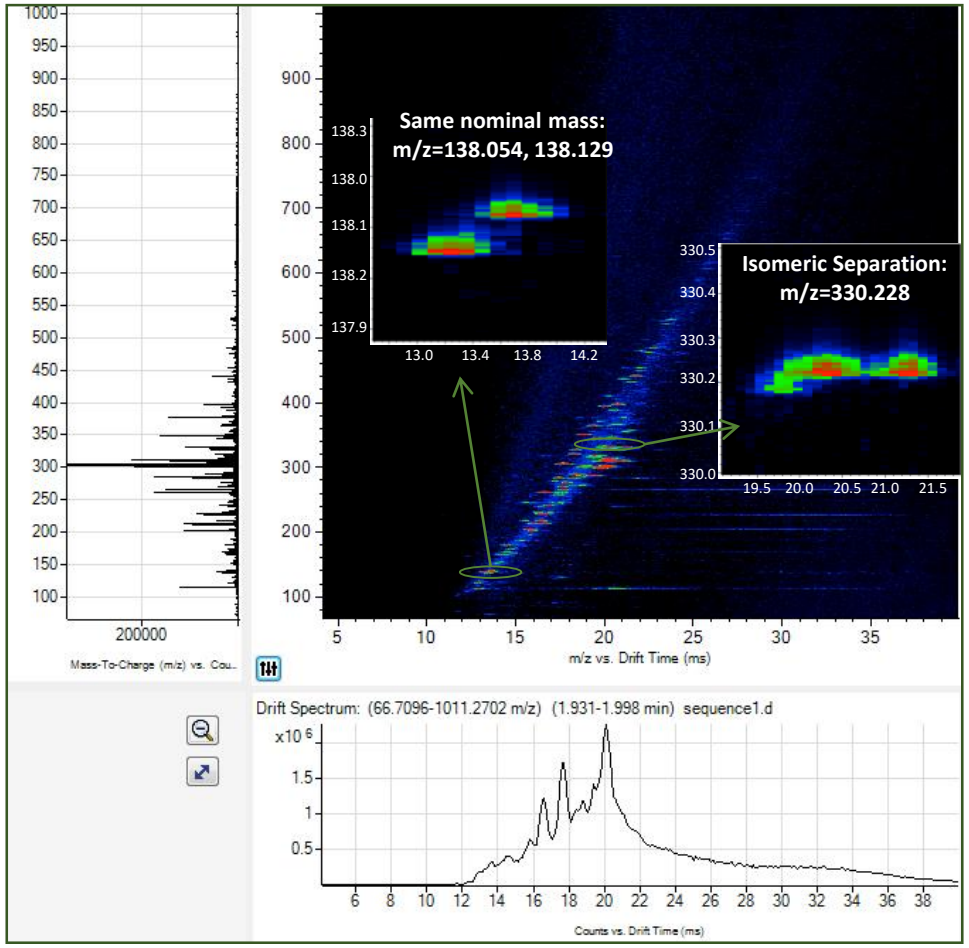
SPE-IMS-MS analyses of biological samples

Metabolites extracted from mouse plasma



~1400 features with S/N > 5

Metabolites extracted from human urine



~1000 features with S/N > 5