Small Molecule Analysis Using Diverse Separation Techniques Coupled to Microelectrospray Mass Spectrometry

Paul Vouros
Barnett Institute and Department of Chemistry
Northeastern University
Outline

1. Capillary LC μESI/MS for Trace level analysis of DNA adducts from in vivo samples

2. Normal bore HPLC μESI/MS applied to drug metabolism problems

3. Ultrafast Analysis Using On-line μESI - Differential Ion Mobility - MS
Part 1: Capillary LC-MS Analysis of DNA Adducts
Virus, Chemical, Radiation → DNA → Tumor Development
## Amount of DNA and Tissue Required to Isolate $1\mu g$ of Adduct at Various Levels of Modification

<table>
<thead>
<tr>
<th>Adduct Level Tissue (1 modification per $10^x$ bases)</th>
<th>Amount of DNA Required</th>
<th>Amount of Tissue Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6$</td>
<td>1g</td>
<td>1 kg</td>
</tr>
<tr>
<td>$10^7$</td>
<td>10g</td>
<td>10kg</td>
</tr>
<tr>
<td>$10^8$</td>
<td>100 g</td>
<td>100kg</td>
</tr>
<tr>
<td>$10^9$</td>
<td>1kg</td>
<td>1,000kg</td>
</tr>
</tbody>
</table>
Heterocyclic Amines

Cooking

Maillard Reaction
(amino acids + sugars + creatine)

Quinoxalines
R = H or CH₃

Quinolines
R = H or CH₃

Pyridines
R₁ = H or CH₃,
R₂ = H, CH₃, or Phenyl
IQ Metabolism

Cytochrome P4501A2

Acetyltransferase

Nitrenium Ion Intermediate

dG-C8-IQ
dG-N^2-IQ

IQ Metabolism
Product Ion Spectra of dG-C8-IQ and dG-N\textsuperscript{2}-IQ
Schematic of Capillary LC-μESI Interface

- Packed capillary column: 365 µm O.D. x 75 µm I.D.
- Fused silica tip: 138 µm O.D. x 29 µm I.D.
- Valco stainless steel microvolume union
- Expanded view of microelectrospray tip

NanoLC flow (300nl/min) from Agilent 1100 Capillary HPLC

Negative pressure sample introduction

Injector

ESI tip XYZ positioner

Sliding rail mount

Sample
Schematic of capillary liquid chromatography system in line with Finnigan TSQ 700 mass spectrometer.
IQ administered by gavage 24 h prior to autopsy

Animals sacrificed with overdose sodium pentobarbital (60 mg/kg ip)

Liver tissue immediately obtained and frozen at –80 °C

Nuclear DNA isolated by Qiagen chromatography and stored at –80 °C in 1 mL water

300 µg DNA aliquot transferred to an incubation vial containing TRIS/MgCl pH 7.6 buffer then spiked with 85 fmole dG-C8-IQ-\textsuperscript{d3}

DNA digested to nucleosides at 37 °C using:
1. Dnase 1
2. Phosphodiesterase
3. Alkaline phosphatase

IQ adducted nucleosides isolated by solid phase extraction

DNA digested to nucleosides at 37 °C using:
1. Dnase 1
2. Phosphodiesterase
3. Alkaline phosphatase

Methanol fraction from SPE evaporated under vacuum

Sample reconstituted with 20 µL 10:90 (v/v%) MSO:water

0.5 µL injected into triple quadrupole MS operating in MRM mode

**SPE protocol:**

**SPE cartridges:**
IST™ Isolute C18(EC)
100 mg/1 mL
pore size (avg.) 54Å

**Conditioning:**
3 mL- methanol
3 mL- 5 mM triethylamine in water pH 3.5 (adj. Using 1M HCl)

**Sample addition:**

**Wash:**
3 mL- 5 mM triethylamine in 10:90 (v/v%) methanol: water pH 3.5
3 mL- water

**Desorption:**
1 mL- methanol
dG-C8-IQ Standard Curve Using Blank Rat Liver DNA

\[ y = 7.41 \times 10^{-3}x - 1.53 \times 10^{-1} \]

\[ R = 0.988 \]

LOQ = 17.5 fmole (s/n 10:1)

LOD = 6.00 fmole (s/n 3:1)

Peak height ratio (analyte/I.S.)

Mass dG-C8-IQ (fmole) added to 300 µg rat liver DNA
Selected Chromatograms of Liver DNA from Rats dosed with IQ at Different Levels

A 0.05 mg/kg

B 0.5 mg/kg

C 1 mg/kg

D 10 mg/kg

E Blank

d$_3$ = 85 fmole

S/N: 16

S/N: 54

S/N: 34

S/N: 69

S/N: 73

S/N: 45

S/N: 305

S/N: 50

0.05 mg/kg

0.5 mg/kg

1 mg/kg

10 mg/kg

Blank
Comparison of dG-C8-IQ Adduct Levels as a Function of IQ Dose Using Different Methodologies

<table>
<thead>
<tr>
<th>IQ Dose (mg/kg)</th>
<th>Animal Code</th>
<th>Mass of dG-C8-IQ (fmole)</th>
<th>adducts/10^8 bases</th>
<th>(A) capillary LC/μESI-MS analysis^a</th>
<th>(B) ^32P-postlabeling analysis^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>Rat 1</td>
<td>31.8 ± 6.7</td>
<td>3.5 ± 0.70</td>
<td>0.05</td>
<td>0.40 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Rat 3</td>
<td>28.4 ± 1.3</td>
<td>3.1 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>Rat 4</td>
<td>75.7 ± 18</td>
<td>8.4 ± 2.0</td>
<td>0.30</td>
<td>1.0 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Rat 5</td>
<td>67.9 ± 22</td>
<td>7.6 ± 2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat 6</td>
<td>62.5 ± 9.8</td>
<td>6.9 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>Rat 7</td>
<td>122 ± 30</td>
<td>14 ± 3.3</td>
<td>1.0</td>
<td>2.0 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Rat 8</td>
<td>90.4 ± 33</td>
<td>10 ± 3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat 9</td>
<td>95.2 ± 7.6</td>
<td>10 ± 0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>Rat 10</td>
<td>351 ± 32</td>
<td>39 ± 3.4</td>
<td>10.0</td>
<td>33 ± 12</td>
</tr>
<tr>
<td></td>
<td>Rat 11</td>
<td>349 ± 0.60</td>
<td>38 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat 12</td>
<td>340 ± 52</td>
<td>37 ± 5.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a (A) dG-C8-IQ adduction level dose-response data determined using capillary LC-mESI-MS.
^b (B) dG-C8-IQ adduction level dose-response data generated during a separate study where the C8-IQ adduct content in each rat liver DNA was determined by ^32P-postlabeling. ^32P-postlabeling value does not take into account 50% labeling efficiency. ^c Mean ± SD of three independent analyses for rats 1-9 and two independent analyses for rats 10-12. ^d Values obtained from three independent analyses ± SD.
• Group 1 aromatic amine carcinogens reported in mainstream cigarette smoke
  • 2- Napthylamine (12 ng / cigarette)
  • 4-Aminobiphenyl (4 ng / cigarette)

dG-C8-ABP Standard Curve

\[ y = 0.132x - 0.324 \]
\[ R = 0.999 \]

LOQ \((s/n \ 10:1) = 0.55 \text{ fmole} = 0.55 / 10^9 = 6.6 / \text{cell}\)

LOD \((s/n \ 3 :1) = 0.17 \text{ fmole} = 0.17 / 10^9 = 2.0 / \text{cell}\)

Note: 20 fmole adducts in 300 µg DNA correspond to \(2 / 10^8 \text{nt}\) and 240/cell.
## Limits of Quantification of 4-ABP dG-Adducts

<table>
<thead>
<tr>
<th>Digest #</th>
<th>RT (min.)</th>
<th>Peak Height</th>
<th>Peak Area</th>
<th>S/N</th>
<th>dG-C8-ABP LOQ (10 x S/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mass (attomole)</td>
</tr>
<tr>
<td>1</td>
<td>28.03</td>
<td>10619370</td>
<td>69541134</td>
<td>21</td>
<td>810</td>
</tr>
<tr>
<td>2</td>
<td>27.78</td>
<td>13638378</td>
<td>98153414</td>
<td>25</td>
<td>680</td>
</tr>
<tr>
<td>3</td>
<td>27.76</td>
<td>10099080</td>
<td>61883210</td>
<td>19</td>
<td>895</td>
</tr>
<tr>
<td>mean</td>
<td>27.86</td>
<td>11452276</td>
<td>76525919</td>
<td>22</td>
<td>795</td>
</tr>
<tr>
<td>SD</td>
<td>0.16</td>
<td>1911009</td>
<td>19117331</td>
<td>3.1</td>
<td>108</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.58</td>
<td>17</td>
<td>25</td>
<td>14</td>
<td>13.6</td>
</tr>
</tbody>
</table>

* dG-C8-ABP peak data following capLC/µESI/MS analysis of the 300 ng in vitro adducted ct DNA digests in figure 5.10. LOQ mass (attomole) calculations based on the samples containing 1.73 fmole dG-C8-ABP. Calculation of # adducts/10^{10} NS based on using 300 µg DNA.
## Quantification of dG-C8-ABP in Human Samples

based on standard curve calculation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gender</th>
<th>Smoker/ Non-Smoker</th>
<th>Age</th>
<th>IS Area</th>
<th>Analyte Area</th>
<th>Analyte/ IS ratio</th>
<th>fmole of dG-C8-ABP</th>
<th>Adducts/ 10^8 nucleosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>F</td>
<td>NS</td>
<td>17</td>
<td>63972</td>
<td>18670</td>
<td>0.29185</td>
<td>&lt; 5</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>F</td>
<td>NS</td>
<td>20</td>
<td>41342</td>
<td>88712</td>
<td>2.1458</td>
<td>72.8</td>
<td>8.10</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>NS</td>
<td>42</td>
<td>49010</td>
<td>0</td>
<td>0.00</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>NS</td>
<td>22</td>
<td>55601</td>
<td>46704</td>
<td>0.83998</td>
<td>20.3</td>
<td>2.26</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>NS</td>
<td>59</td>
<td>52883</td>
<td>12428</td>
<td>0.23501</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>M</td>
<td>NS</td>
<td>37</td>
<td>55423</td>
<td>764139</td>
<td>13.787</td>
<td>540</td>
<td>60.1</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>S</td>
<td>41</td>
<td>50777</td>
<td>0</td>
<td>0.00</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>F</td>
<td>S</td>
<td>63</td>
<td>51422</td>
<td>86159</td>
<td>1.6755</td>
<td>53.9</td>
<td>6.00</td>
</tr>
<tr>
<td>62</td>
<td>F</td>
<td>S</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>no data</td>
<td></td>
</tr>
<tr>
<td>152</td>
<td>M</td>
<td>S</td>
<td>23</td>
<td>53680</td>
<td>18258</td>
<td>0.34013</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>159</td>
<td>M</td>
<td>S</td>
<td>24</td>
<td>49554</td>
<td>272996</td>
<td>5.5091</td>
<td>208</td>
<td>23.1</td>
</tr>
<tr>
<td>163</td>
<td>M</td>
<td>S</td>
<td>23</td>
<td>20932</td>
<td>12366</td>
<td>0.59077</td>
<td>10.3</td>
<td>1.15</td>
</tr>
</tbody>
</table>
Conclusions

• Capillary LC – μESI / MS well suited for trace level analysis in general and of DNA adducts in particular

  • Potential Drawbacks:
  • Poor loading capacity
  • Longer analysis times
  • Need for better cleanup
The Link Between DNA Modification and Cancer

ULTIMATE GOAL

Correlate:

DNA Adduct Formation with Mutagenicity and Gene Expression in human lymphoblast cells dosed with Benzo[a]pyrene and 4-Aminobiphenyl
Exposure: TK6 cells dosed at various levels of OH-AABP & BPDE

DNA adducts: Analyzed dose levels of 65%, 80% & 95% for OH-AABP

Cell

Expression Arrays: Analyzed dose levels for both compound: 65%, 80%, & 95% survival rate

Mutation & Toxicity: Analyzed all cells dosed at various levels
Gene Expression Experimental Design

Amplify cDNAs and array in multiwell format

Control Cell Population
Extract mRNA
\[ \text{cDNA} \]
label w/ green fluor
Co-hybridize to array
Printed array
Scan

Exposed Cell Population
Extract mRNA
\[ \text{cDNA} \]
label w/ red fluor

Data analysis
Part 2: Outline

✓ Background
✓ Design and construction of post-column nano-splitting device
✓ Applications
  ✓ Linearity and dynamic range investigations
  ✓ *In vitro* Indinavir metabolism in rat hepatocyte incubations
  ✓ *In vivo* metabolism studies with simultaneous C14 detection: detection of metabolites from rat urine
Smaller Droplets in Nanospray (~0.2mm) Compared to ESI Drops (~1mm)

- Desolvate easier; do not require nebulizing gas
- Contain a lower number of molecules, which decreases analyte clustering and increases ionization efficiency
- Have a higher surface area - to - volume ratio, allowing more analyte molecules to reside on the droplet surface
- Have 20-25x higher charge - to - volume ratio (taking into account the current is approximately 1/4 less in nano ESI)
- Allow the spray tip to be placed in close proximity to the orifice, increasing ion transmission efficiency without concern for arcing
- Tolerate higher salt concentrations
- Allow for minimal sample clean-up
Detailed Schematic of Nanosplitter Interface

- **High Voltage**
- **Stainless Steel Split Arm**
- **Fused Silica Tip**
  - O.D. 360 μm, I.D. 75 μm
  - Pulled to a 10 μm coated tip
- **Valco stainless steel microvolume union**

**Flow Rates**
- **LC Flow Rate**: 200 μl/min
- **MS Flow Rate**: 0.2 μl/min

**Components**
- **Restrict Needle Valve**
- **Union**
- **PEEK μSheath**

**Notes**
- Fused Silica Tip
  - O.D. 360 μm, I.D. 75 μm
  - Pulled to a 10 μm coated tip
Why Do We Think Concentric Splitting Works?

- **Waste or Collection**
- **Needle Valve** - Adjusts Back Pressure
- **Split Point**
- **Bulk Flow from HPLC**
- **Stainless Steel**
- **HV Power**
- Fused silica tip
  - 360 μm O.D.
  - 75 μm I.D.
  - Pulled to a 10 μm tip
- Metal coated

**ESI Manifold and Heated Capillary**
Investigation of Linearity, Dynamic Range and Sensitivity of the Nano-splitter
Fragmentation of Glyburide

2nd Generation sulfonylurea with hypoglycemic activity
C_{23}H_{28}ClN_{3}O_{6}S, MW = 494

* Internal Standard is a Proprietary Structural Analogue
Interday Absolute Analyte Area Curves for Glyburide Plasma Samples - Standard and Nanosplitter Interfaces

**Calibration Curves**

**Nanosplitter:**
\[ y = 0.0019x + 0.051, \text{ LOQ} = 0.05\text{ng} \]
\[ R^2 = 0.9984, n = 6 \]

**Standard Interface:**
\[ y = 0.0014x + 0., \text{ LOQ} = 0.25\text{ng} \]
\[ R^2 = 0.9994, n = 4 \]
Metabolism Studies: *In vitro* Incubation of Indinavir and Rat Hepatocytes
Hydroxylated Indinavir Metabolites from Rat Hepatocyte Incubations

A: 200 µl/min into MS

B: 0.1 µl/min into MS
Metabolism Studies: *In vivo* Rat Urine Experiments with Simultaneous C14 detection
Drug Metabolite Group Set-up for Nanosplitter during GSK visit - April 2002

- PAL Autosampler
- Agilent 1100 HPLC Pump
- 4.6x250mm HPLC column
- Microtee (1:9 split)
- Divert Valve
- Radio detector
- Waste
- Finnigan TSQ7000 Triple Quadrupole Mass Spectrometer
On-line Radiometric Traces for Metabolites from Rat Urine using Standard and Nanosplitter Interfaces

**Standard Interface**

8.11 x 10^-4

**Nanosplitter Interface**

8.30 x 10^-4
Parent and Metabolite Full Scan MS Traces for Standard Interface

- **3.54 \times 10^5**  
  \( m/z = 541 \)  
  Area: 6427176

- **2.38 \times 10^5**  
  \( m/z = 557 \)  
  Area: 3468047

- **9.03 \times 10^4**  
  \( m/z = 276 \)  
  Area: 569619  
  Area: 135183

- **3.90 \times 10^4**  
  \( m/z = 292 \)  
  Area: 836717  
  Area: 476587

- Area: 200343

**Relative Abundance**

**Time (min)**
Parent and Metabolite Full Scan MS Traces for Nanosplitter Interface

- $1.18 \times 10^6 \quad m/z = 541$
  - Area: 20193239

- $1.12 \times 10^6 \quad m/z = 557$
  - Area: 20761556
  - Area: 4943348

- $2.45 \times 10^6 \quad m/z = 276$
  - Area: 36430674

- $2.90 \times 10^5 \quad m/z = 292$
  - Area: 4383879
  - Area: 2640134
  - Area: 1021121
  - Area: 1724119
$m/z=292$ Traces for Standard and Nanosplitter Interfaces

3.90 x 10^4
Standard Interface

2.90 x 10^5
Nanosplitter Interface
## Summary of Rat Urine Metabolism Studies

<table>
<thead>
<tr>
<th>Mass</th>
<th>Mass Change</th>
<th>Metabolism Pathway</th>
<th>Std Interface</th>
<th>NanoSplitter</th>
<th>Change in Ret Time</th>
<th>% Signal Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak RT</td>
<td>Peak Area</td>
<td>S/N</td>
<td>Peak RT</td>
</tr>
<tr>
<td>541</td>
<td>Parent</td>
<td>51.41</td>
<td>6427176</td>
<td>223</td>
<td>52.25</td>
<td>20193239</td>
</tr>
<tr>
<td>557</td>
<td>16 oxidation</td>
<td>37.87</td>
<td>569619</td>
<td>69</td>
<td>38.67</td>
<td>4943348</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.78</td>
<td>3468047</td>
<td>295</td>
<td>39.54</td>
<td>758064</td>
</tr>
<tr>
<td>276</td>
<td>-265 hydrolysis</td>
<td>23.63</td>
<td>836717</td>
<td>110</td>
<td>24.43</td>
<td>36430674</td>
</tr>
<tr>
<td>292</td>
<td>-249 hydrolysis</td>
<td>13.33</td>
<td>200343</td>
<td>37</td>
<td>14.00</td>
<td>2640134</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.94</td>
<td>476587</td>
<td>63</td>
<td>16.68</td>
<td>4383879</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.58</td>
<td>64219</td>
<td>17</td>
<td>18.38</td>
<td>1724119</td>
</tr>
</tbody>
</table>
Part 2: Conclusions

- Development of a nanosplitting interface that allows use of “normal” bore LC with micro electrospray
- Allows exploitation of beneficial large column capacity in LC and increased sensitivity in micro electrospray
- Recovery of 99.9% of the sample for collection or further analysis
- Demonstration of:
  - Dynamic Range
  - Higher sensitivity
  - Lower detection limits
  - Reduction of ion suppression
Acknowledgements

Northeastern University
Eric Gangl
John Soglia
Elaine Ricicki
Christine Andrews
Wendy Luo
Jimmy Flarakos

Fred Hutchinson Cancer Ctr
Helmut Zarbl

Nestle Ltd / NCTR
Rob Turesky
Axel Pähler
Fred Kadlubar

GlaxoSmithKline
Neil Spooner
Meg Annan
C. P. Yu
Eric Yang
Neal Spooner

Support
NIH / NCI
PART III

Separations Using Differential Ion Mobility
DMS Operation Principle

Ionization Source

Sample in ~ ~ ~ ~ ~ ~

Ion Trajectories

DMS or FAIMS is a tunable ion filter

RF electric field

Compensation electric field

Gas Flow → Detector

E_{RF}(t) → t

E_c → t
Differential Mobility separation within the Sionex DMS sensor

- Rapid separation/detection (msec)
- Internal + and – ion electrometer detectors
- 25¢ Quarter size

Mass: $m_1 = m_2$

Cross sectional area: $m_1 > m_2$
Custom nanoESI-DMS-MS system

- Incorporates a modified Sionex Co. SDP-1 DMS sensor
- Micromass ZQ mass spectrometer
- Custom made nanospray source
DMS-MS 3D Spectra of Butanone
APCI in ambient air

• Shows orthogonality of separations
• Tune $V_c$ and detect by MS
• E.g., m/z 73 [M+H]$^+$ and also $(\text{H}_2\text{O})_4\text{H}^+$

Courtesy of E. Nazarov, SIONEX,
Example DMS dispersion plot (a) and extracted DMS spectra at Rf = 1000 V (b), selected Rf and Vc point mass spectra (c and d)
Demonstration of Mass Spectral Signal/Noise Improvement through DMS Ion Separation

- Mass Spectra of Lacto-N-fucopentaose I (LNFP I) sample (oligosaccharide with M.W. 854) with and without DMS separation

A: No DMS separation

B: Mass Spectrum with DMS separation

C: Mass Spectrum with DMS separation, showing an increase in signal/noise ratio with Vc = -8.7
Study 1: Investigation into rapid peptide separation and quantitation capabilities via direct infusion nanoESI-DMS-MS

- DMS peptide separation?
- Can drift gas modifiers improve separation?
- Hurdle: peptide aggregate/cluster ion formation
  - nanoESI formation of 17mer$^{4+}$ and 19mer$^{5+}$ aggregate ions of leucine enkaphalin in high abundance.\(^1\)
  - ESI formation of heteroaggregate ions in addition to homoaggregate ions from peptide mixture \(^2,3\)
  - May contribute to high m/z background ions observed in ESI-MS peptide analysis \(^2\)

---


Mixture of Angiotensin, Glu-Fib B, and Neurotensin 0.05 mg/ml each

A, B - DMS off, no modifier; C-G 8000 ppm 2-butanol

E – G Mass spectra at selected Vc points (Rf = 1200)
Ultra-Rapid Quantitation via Selected Ion-Rapid Vc Scanning Platform

- Rf held constant, and Vc rapidly scanned (<10 sec.) and selected MS ion signal monitored
- Match peak apex Vc to standard for specificity and integrate peak area for quantification
- repeat injections of six-peptide mixtures containing 0, 2, 10 and 25 µg/ml angiotensin

<table>
<thead>
<tr>
<th>Amount Quantified</th>
<th>% RSD</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8 µg/ml</td>
<td>3.6%</td>
<td>90%</td>
</tr>
<tr>
<td>9.1 µg/ml</td>
<td>2.5%</td>
<td>91%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2 µg/ml Ang. Spiked into mixture of 7 peptides (10 nmol/ml)</th>
<th>10 µg/ml Ang. Spiked into mixture of 7 peptides (10 nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>scan #</td>
<td>peak area</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
<td>202817</td>
</tr>
<tr>
<td>2</td>
<td>220863</td>
</tr>
<tr>
<td>3</td>
<td>213123</td>
</tr>
<tr>
<td>4</td>
<td>201225</td>
</tr>
<tr>
<td>5</td>
<td>203341</td>
</tr>
<tr>
<td>6</td>
<td>208827</td>
</tr>
<tr>
<td>average</td>
<td>208366.0</td>
</tr>
<tr>
<td>std dev</td>
<td>7563.8</td>
</tr>
<tr>
<td>%RSD</td>
<td>3.6%</td>
</tr>
<tr>
<td>Amount Quantified</td>
<td>1.8 µg/ml</td>
</tr>
<tr>
<td>% recovery</td>
<td>90%</td>
</tr>
</tbody>
</table>
Linear regression of angiotensin concentration (µg/ml) vs. response (peak area ratio of angiotensin/internal std.) spiked into six peptide mixture. Inlay plot shows zoomed-in view of the four low concentration and blank solution data points. Scan time: 10 sec/point

<table>
<thead>
<tr>
<th>angiotensin fragment concentration (µg/ml)</th>
<th>peak area ratio (angiotensin / internal std.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0040</td>
</tr>
<tr>
<td>0.02</td>
<td>0.0086</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0248</td>
</tr>
<tr>
<td>0.2</td>
<td>0.0417</td>
</tr>
<tr>
<td>0.4</td>
<td>0.0906</td>
</tr>
<tr>
<td>2</td>
<td>0.4510</td>
</tr>
<tr>
<td>10</td>
<td>2.0944</td>
</tr>
<tr>
<td>50</td>
<td>11.5238</td>
</tr>
</tbody>
</table>

$R^2 = 0.9997$
Using this platform for ultra-high throughput quantitative analysis

• 0.5 – 5 sec. sample analysis times appear reasonable to achieve

• Incorporate an automated sample handling/nanospray system to improve quantitative reproducibility by:
  – Eliminating carryover
  – Reducing capillary clogging
  – Providing reproducible flow and tip position

• Nanospray conditions provide:
  – Improved sensitivity
  – Reduced ion suppression effects
  – Reduced sample consumption

Nanomate
by Advion Biosciences
Conclusions

• Demonstration of improved DMS peptide separation via selected drift gas modifier

• Mechanistic insight into peptide aggregate ion de-clustering and improved DMS separation

• Demonstrated feasibility of selected ion-rapid Vc scanning quantitation platform for various high-throughput applications

• Future work to combine an automated sample handling/nanospray device with DMS-MS system for improved accuracy and throughput
Acknowledgments

Daren Levin

• Department of Chemistry and Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA

• Sionex Corporation, Bedford, MA
  – Raanan A. Miller (Chief Technology Officer, Founder)
  – Erkinjon G. Nazarov (Chief Scientist)
  – James C. Morris (Principal Investigator)