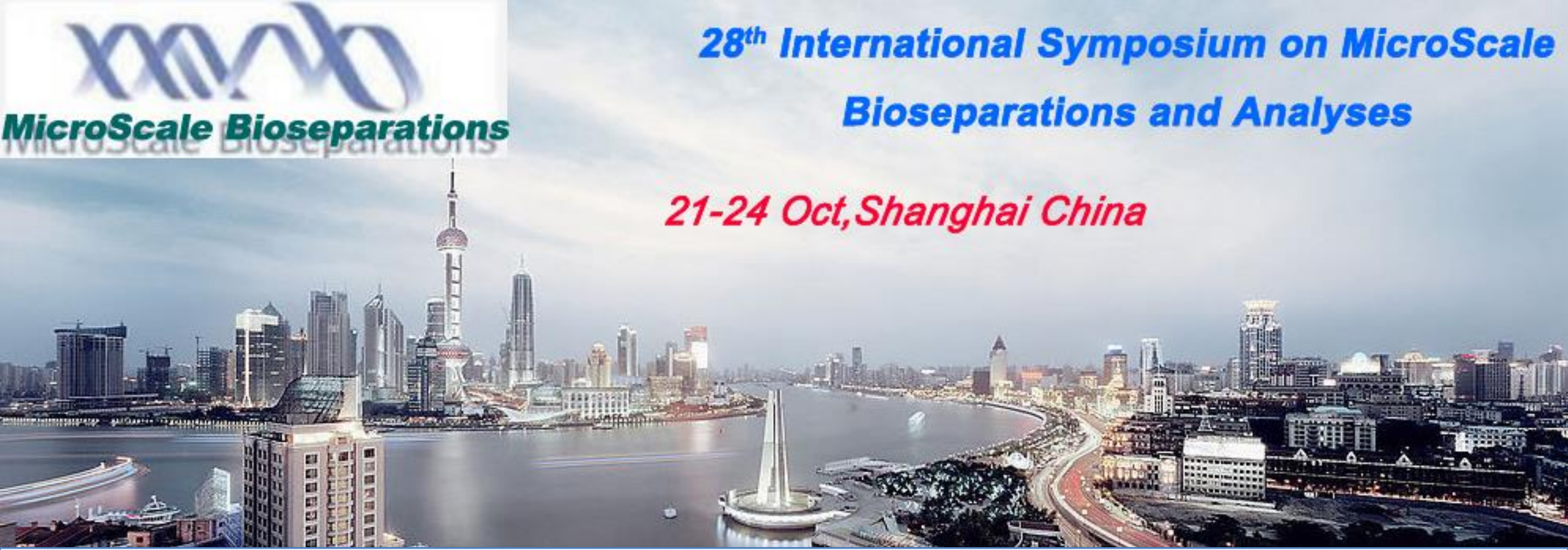


CE-MS; New Developments and Applications

“to sheath or not to sheath; that is the question!”

Gerard Rozing[1], Christian Wenz[2] and Suresh Babu CV[3]

[1]ROZING.COM Consulting, Karlsruhe, Germany

[2]Agilent Technologies, Waldbronn, Germany

[3]Agilent Technologies India Pvt. Ltd., Bangalore, India

CE-Renaissance - New Application Areas*

- CE has become a vital methodology in R&D and production of (bio)pharmaceuticals
  - Recombinant proteins and biogenerics (purity, equivalence, authenticity) in particular glycan profiles, charge and agglomeration studies
- Safety and abuse of (bio)pharmaceuticals
  - Counterfeited small molecule drugs and (bio)pharmaceuticals
  - Blood doping (porcine hemoglobin and hemoglobin oxygen carriers)
  - Contamination of raw materials (e.g heparin case)
- Safety and authenticity of food
  - Counterfeited food (e.g. fish, food supplements)
  - Sterility testing and micro-organism contamination
- Molecular Diagnostics
  - E.g. glycosylation of transferrin for detection of chronic alcohol abuse
- Versatile, robust coupling with MS
  - Metabolomics & Biomarker Discovery
- Kinetic measurements (reaction and separation in a tube!)
  - Determination of physical and chemical molecular properties
  - Enzyme Mediated Molecular Assays (EMMA, Regnier et al.), bio-molecular interactions by affinity ACE, Kinetic Capillary Electrophoresis (KCE, S. Krylov et al.)

*K. Altria, LCGC Chromatography Online, The Column, Vol. 6, Issue 20

New Application Areas for CE

- CE has become a key methodology in R&D and production of biopharmaceuticals
  - Recombinant proteins and biogenerics (purity, equivalence, authenticity)
- Safety and abuse of (bio)pharmaceuticals
  - Counterfeited small molecule drugs and biopharmaceuticals
  - Blood doping (porcine hemoglobin and hemoglobin oxygen carriers)
  - Contamination of raw materials (heparin)
- Authenticity and safety of food
  - Counterfeited food (e.g. fish)
  - Pathogen testing
- Molecular Diagnostics
  - E.g. glycosylation of transferrin for detection of chronic alcohol abuse
- Versatile, robust coupling with MS
  - Metabolomics & Biomarker Discovery
  - New developments in interfacing technology
- Kinetic measurements (reaction and separation in a tube!)
  - Determination of physical and chemical molecular properties
  - Enzyme Mediated Molecular Assays (EMMA, Regnier et al.), bio-molecular interactions by affinity ACE, Kinetic Capillary Electrophoresis (KCE, S. Krylov et al.)

Requirements Interfacing CE by ESI with MS

- Both the CE and the ESI process require a stable electrical contact for current return
- Cope with different current strengths and fields
  - µA for CE, nA for electrospray
  - CE up to 30 kV, ES up to 3 kV, field direction
- Match the CE electrolyte with the ESI process and vacuum mass analysis
  - Volatile buffers (sub-optimal for CE)
- In principle sufficient flow to establish a stable electro-spray
  - Sheath solvent

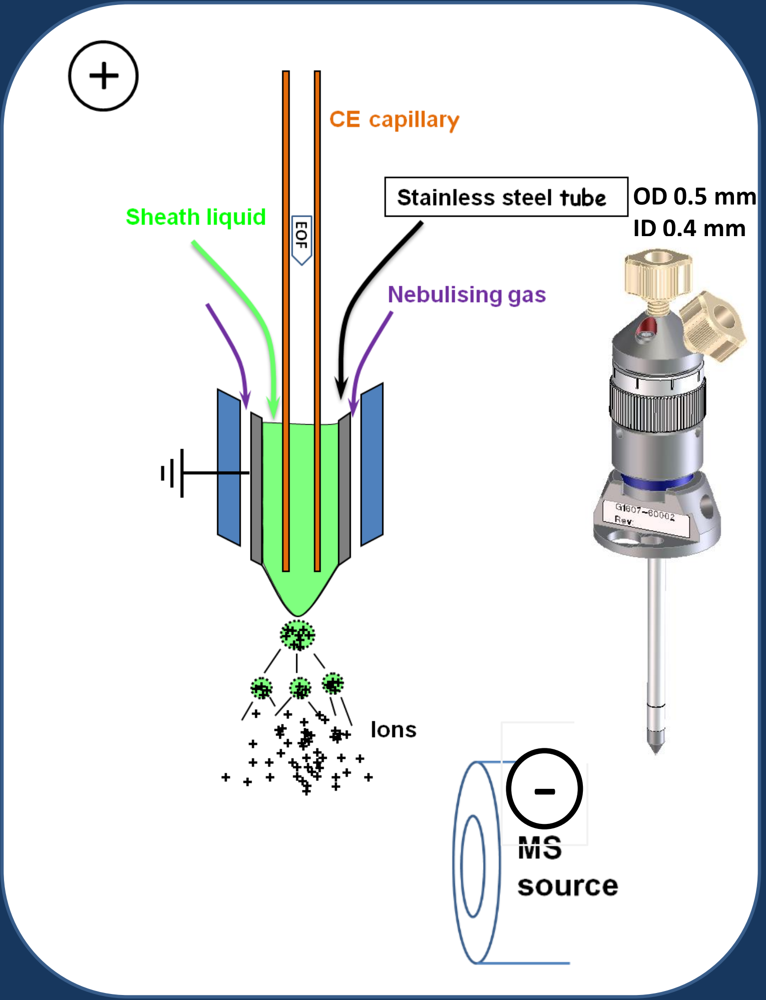
Sheath Liquid Interface for CE-MS – Triple Tube Design*

Sheath solvent is added to the CE effluent at a rate of typically 1 - 5 µL/min. Spray becomes independent of EOF

Spray needle (gray) is grounded. Common ground for CE and ESI. Bubbles are transported out

Sheath solvent composition dominates electrospray ionization chemistry

Orthogonal configuration let neutrals & big droplets pass



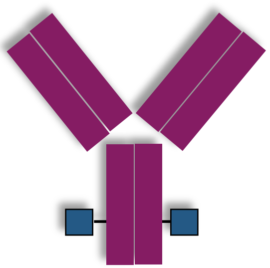
* R.D. Smith et al, Anal. Chem. 60, 1948 (1988)

Glycopeptide Analysis of Antibodies by Capillary Electrophoresis and Q-TOF Mass Spectrometry*

Glycosylation of monoclonal antibodies (mAb) is one of the common post translation modifications. The glycan moieties have a key role in immunogenicity, effector function efficacy, and clearance of the mAbs. Currently mAb are increasingly being used for therapeutics. Therefore, rapid monitoring of mAb glycan status is of great importance.

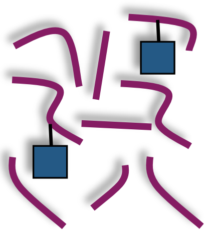
*Contributed by Suresh Babu CV

Experimental Setup



mAb

Trypsin



Peptide digest

CE-MS

CE-MS/MS

- Peptide Mapping
- Sequence confirmation
- Protein coverage
- Glycopeptide analysis
- Glycan modification

Glycan moiety

CE-MS Experimental Conditions

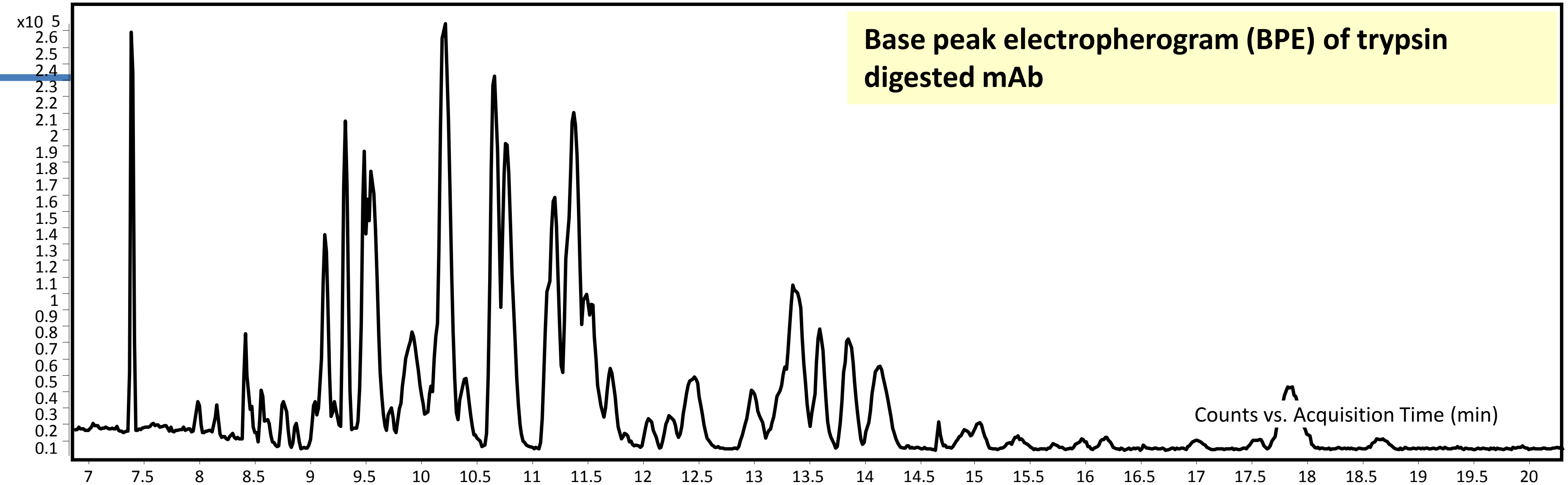
Capillary Electrophoresis (CE)

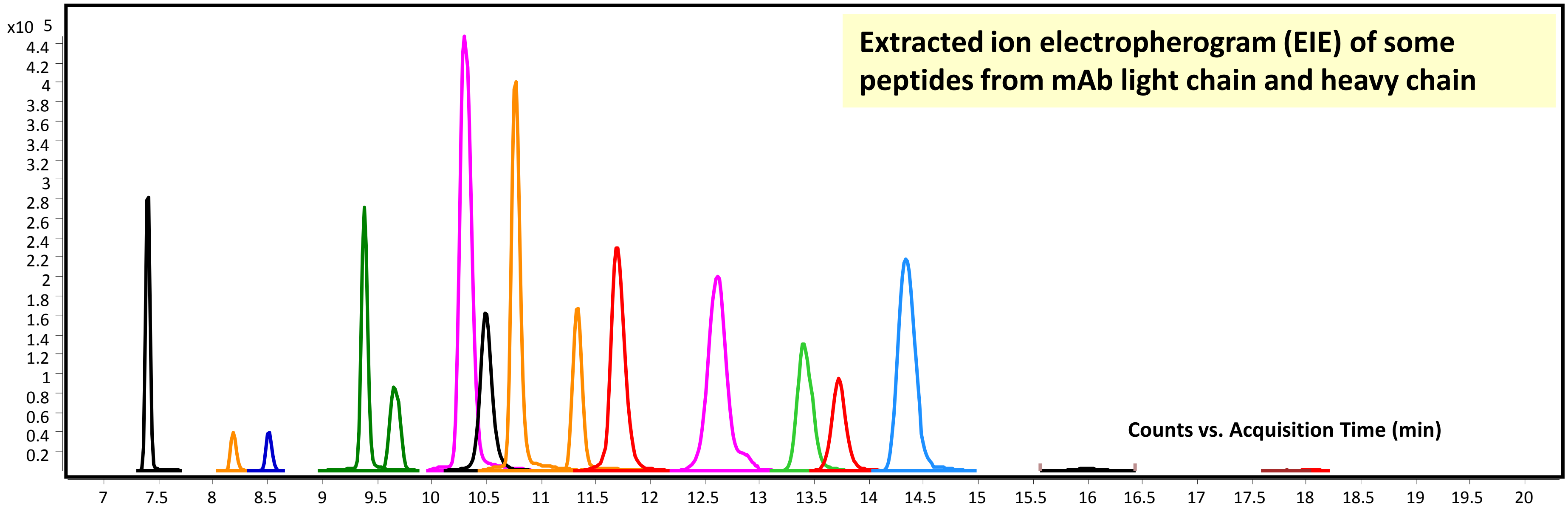
CE:	7100 CE
Sample:	mAb digest
Injection:	10s @ 50 mbar (~0.4pmoles)
Capillary:	Polyvinyl alcohol coated, total length 60 cm, 50 µm ID
Buffer:	2% acetic acid
Voltage:	27 kV
Extr. pressure:	10mbar
Temperature:	20° C

Mass Spectrometry (MS)

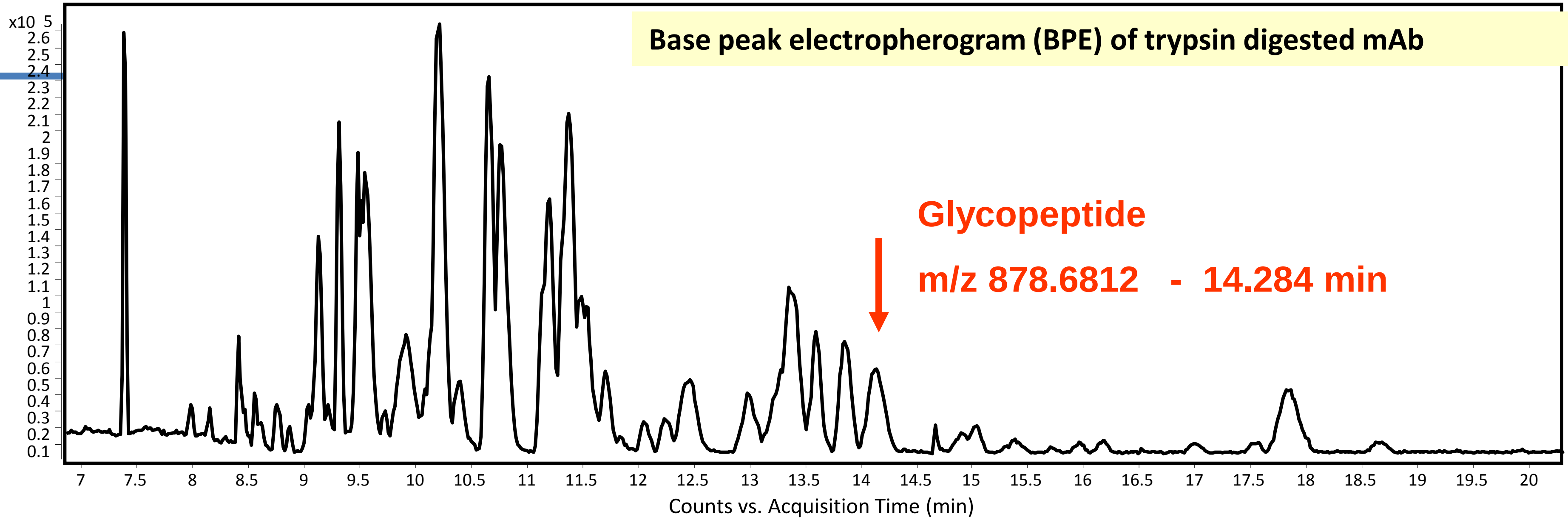
MS:	6520 Q-TOF
Ionization mode:	ESI
Acquisition mode:	MS (mass range 300-3200 m/z)
Sheath liquid:	0.5 % acetic acid in 50 % methanol, 4 µL/min
Drying gas flow:	5 L/min
Nebulizer:	10 psi
Drying gas temp:	150 °C
Fragmentor:	175 V
Vcap:	3500 V
Accu time/rate :	333.3 ms/spectrum and 3 spectra/s
MS/MS:	automatic, no masses excluded
Precursor Threshold:	1000
Isolation width:	~4m/z

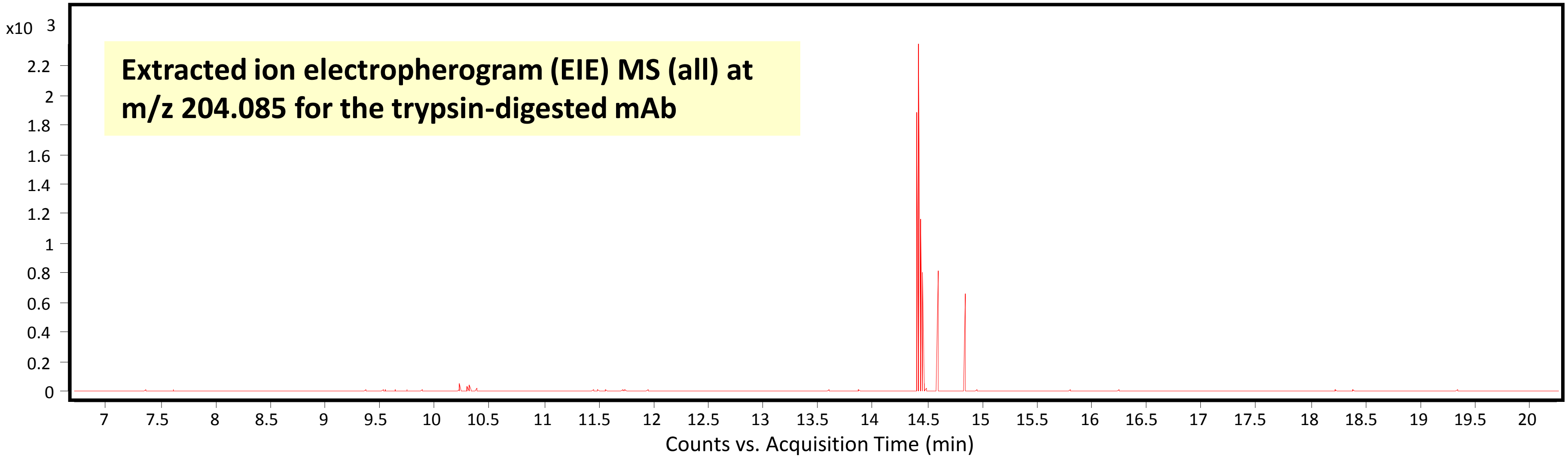
CE-MS of Trypsin Digested mAb





CE-MS and MS/MS of Glycopeptide





Sheath Liquid Interface for CE-MS – Compromises

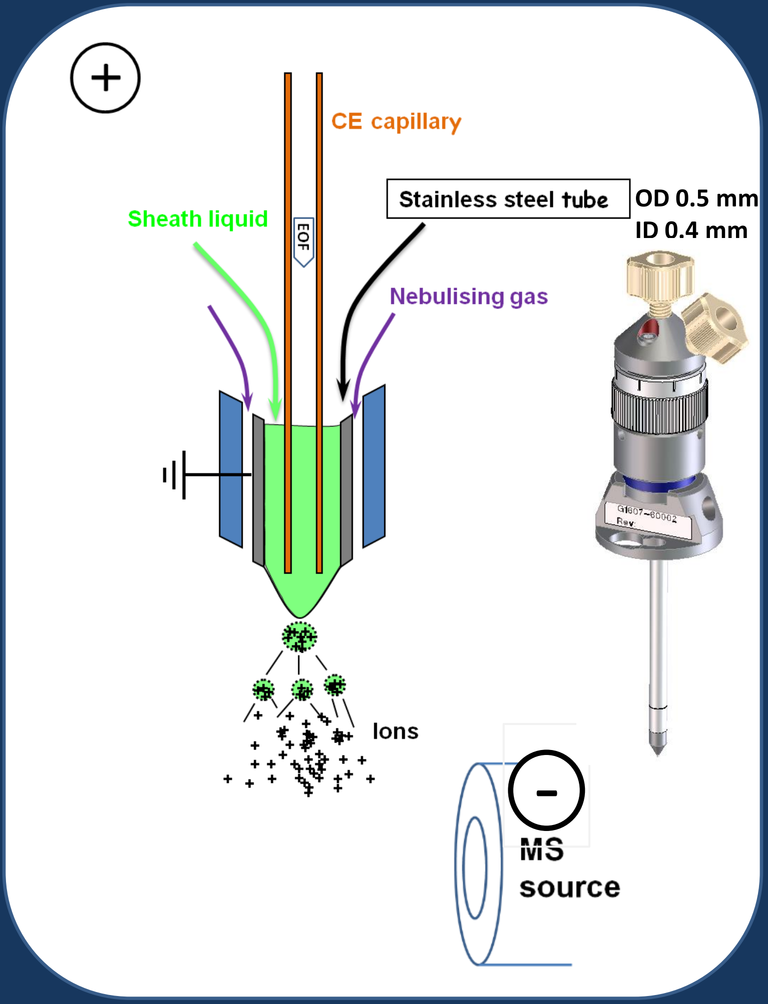
Sheath solvent is added to the CE effluent at a rate of typically 1 - 5 µL/min. Spray becomes independent of EOF

Spray needle (gray) is grounded. Common ground for CE and ESI. Bubbles are transported out

Sheath solvent composition dominates electrospray ionization chemistry

Orthogonal configuration let neutrals & big droplets pass

Sensitivity is compromised!!
➔ Concentration sensitive detection!
➔ Dilution 5 - 50x with the sheath solvent
➔ Higher flow rate compromises nano-electrospray
Pneumatic assistance required to establish the spray
➔Hydraulic flow observed (“venturi” effect)

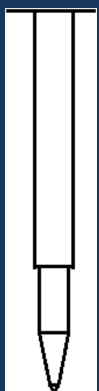


Approaches to Improve Sensitivity of CE-MS

- Improve interfacing
  - Geometry of spray needle
  - Conventional ESI vs. Jetstream IF
- Improved ion transfer
- Interfaces without sheath solvent flow

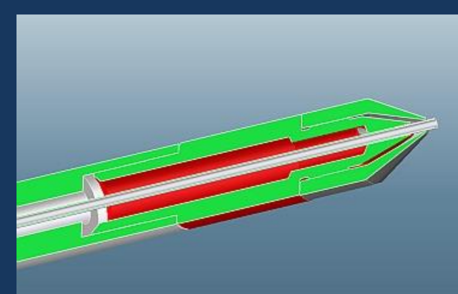
Improved Spray Needle Assembly

"Old"



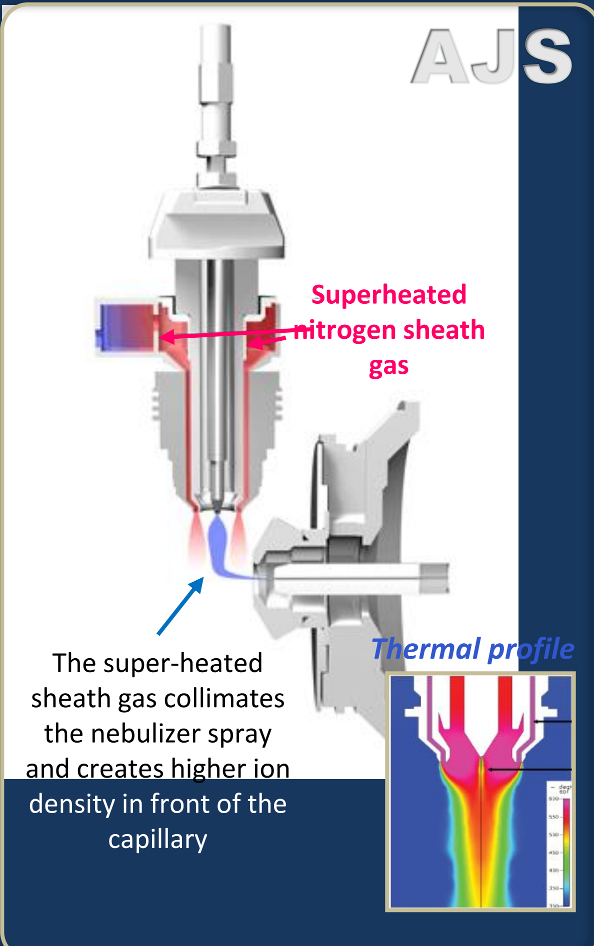
"Improved"

- Needle Tip Geometry
- Fix spray needle alignment with outer tube
- Length (1 mm shorter!)

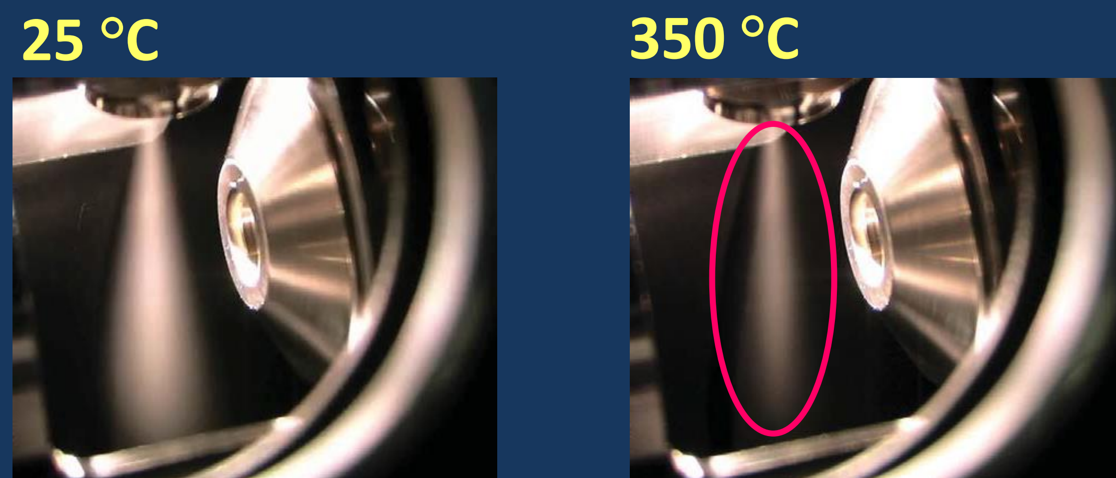


ESI-MS | Agilent Jet Stream technology

Agilent Jet Stream (AJS) thermal gradient focusing technology increases sensitivity of standard column ESI, LC-MS by enhancing de-solvation and spatial focusing of ions



✓ **LC-MS** ? **CE-MS**

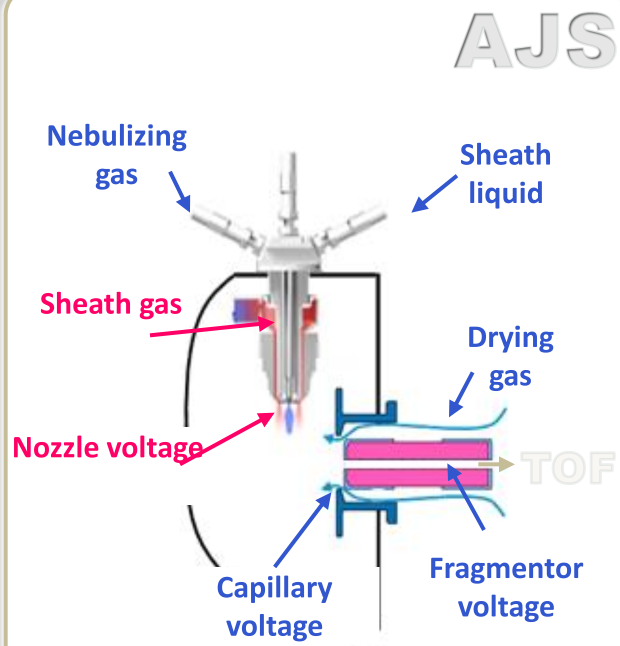


Agilent Jet Stream Thermal Gradient Focusing Technology, Technical Note 5990-3494EN (2009)

Nebulizer in two versions:
HPLC (dual tube), CE (triple tube with modified spraying needle)

CE-ESI-MS | AJS source parameters

Determination of AJS parameters *via* compounds infusion (50 µg/mL in BGE, P = 100 mbar)



Shared with conventional ESI sources

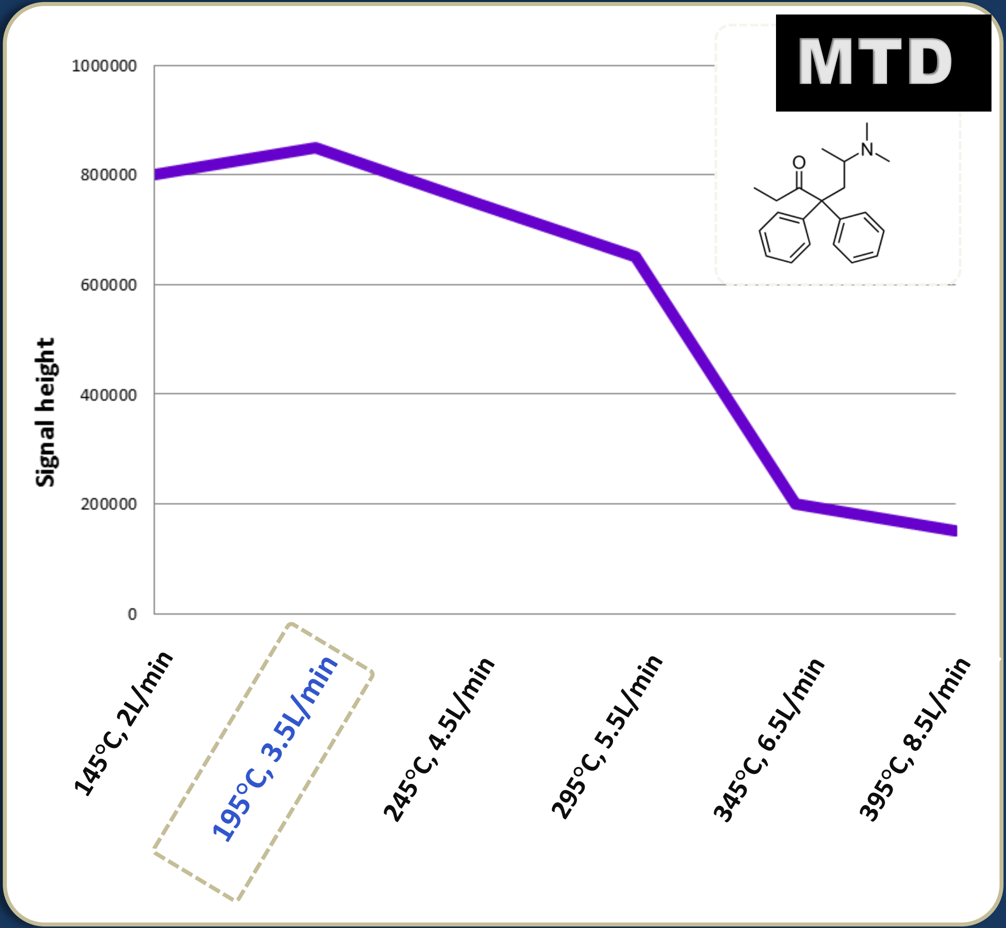
AJS source only

Drying gas temperature	250 °C
Drying gas flow rate	4 L/min
Nebulizing gas pressure	4 psi
Fragmentor voltage	150 V
Sheath liquid flow rate	3 µL/min
Capillary voltage	+1500 V
Nozzle voltage	+2000 V
Sheath gas temperature	195 °C
Sheath gas flow rate	3.5 L/min

LC-MS		CE-MS
350°C	195°C	
11 L/min	3.5 L/min	

CE-ESI-MS | Sheath gas conditions

Sheath gas flow rate and temperature



SHEATH GAS

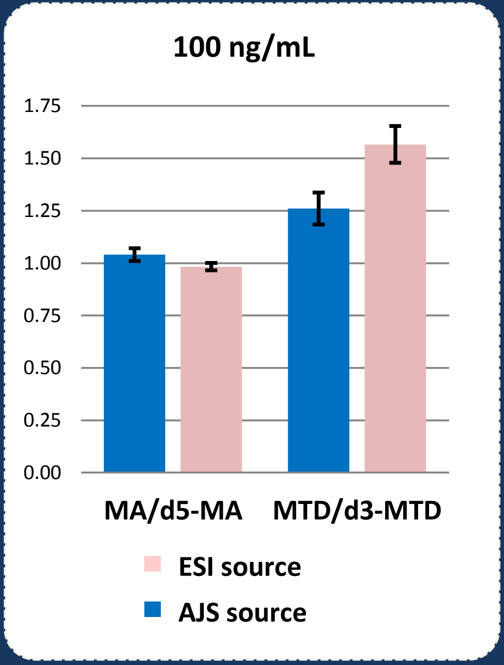
⬀ Sensitivity at ≤ 200°C

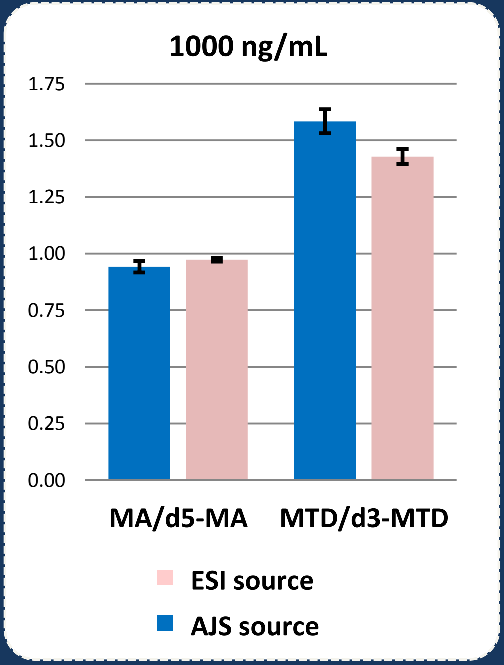
⬀ Sensitivity at low flow rate

CE effluent : 1 – 10 µL/min

LC effluent : 50 – 2500 µL/min

CE-ESI-MS | Comparison of standard ESI vs AJS



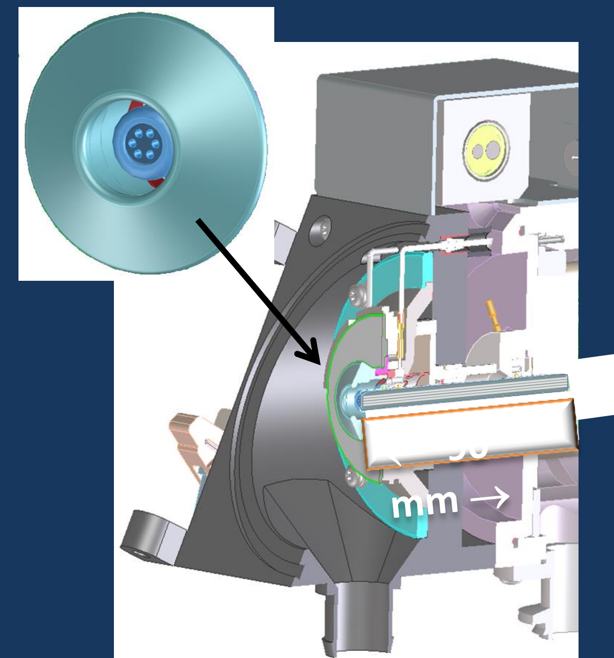


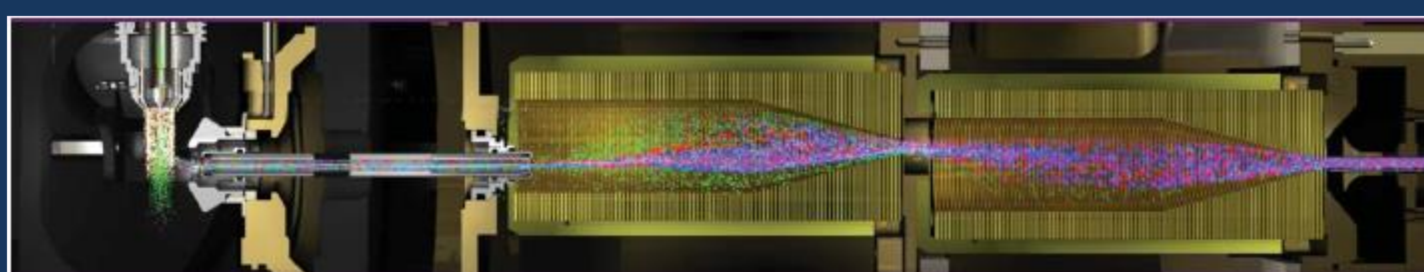
✓ **AJS source ≈ ESI source**

(with internal standard correction)

Improved Ion Transfer Technology

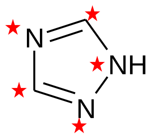
- Hexabore inlet capillary
  - Permeability equal, 6x higher flow
- Dual ion funnel (DIF) technology



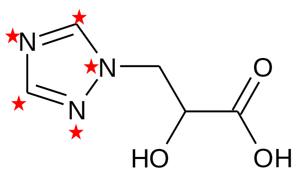


CE/QQQ Analysis of Triazole Metabolites

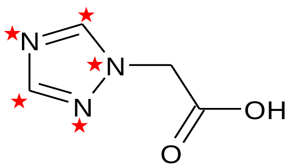
The goal of this study was to determine whether triazole and metabolites (below) could be detected using CE/QQQ and at what level in standard and in matrix. The (★) indicates the position of labeling for internal standards.



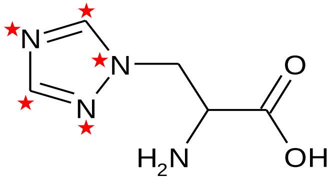
1,2,4-Triazole
Molecular Formula: $C_2H_3N_3$
Molar Mass: 69.03 g/mol
MRM: 70/43 $[M+H]^+$



1,2,4-Triazole Lactic Acid
Molecular Formula: $C_5H_7N_3O_3$
Molar Mass: 157.05 g/mol
MRM: 158/70 $[M+H]^+$



1,2,4-Triazole Acetic Acid
Molecular Formula: $C_4H_5N_3O_2$
Molar Mass: 127.04 g/mol
MRM: 128/70 $[M+H]^+$



1,2,4-Triazole Alanine
Molecular Formula: $C_5H_8N_4O_2$
Molar Mass: 156.06 g/mol
MRM: 157/70 $[M+H]^+$
(157/88) $[M+H]^+$

★

CE/QQQ analysis of Triazole Metabolites

QQQ MS conditions

Ion Mode:	Jetstream IF, improved spraying needle**, positive**

Jet Stream ESI conditions

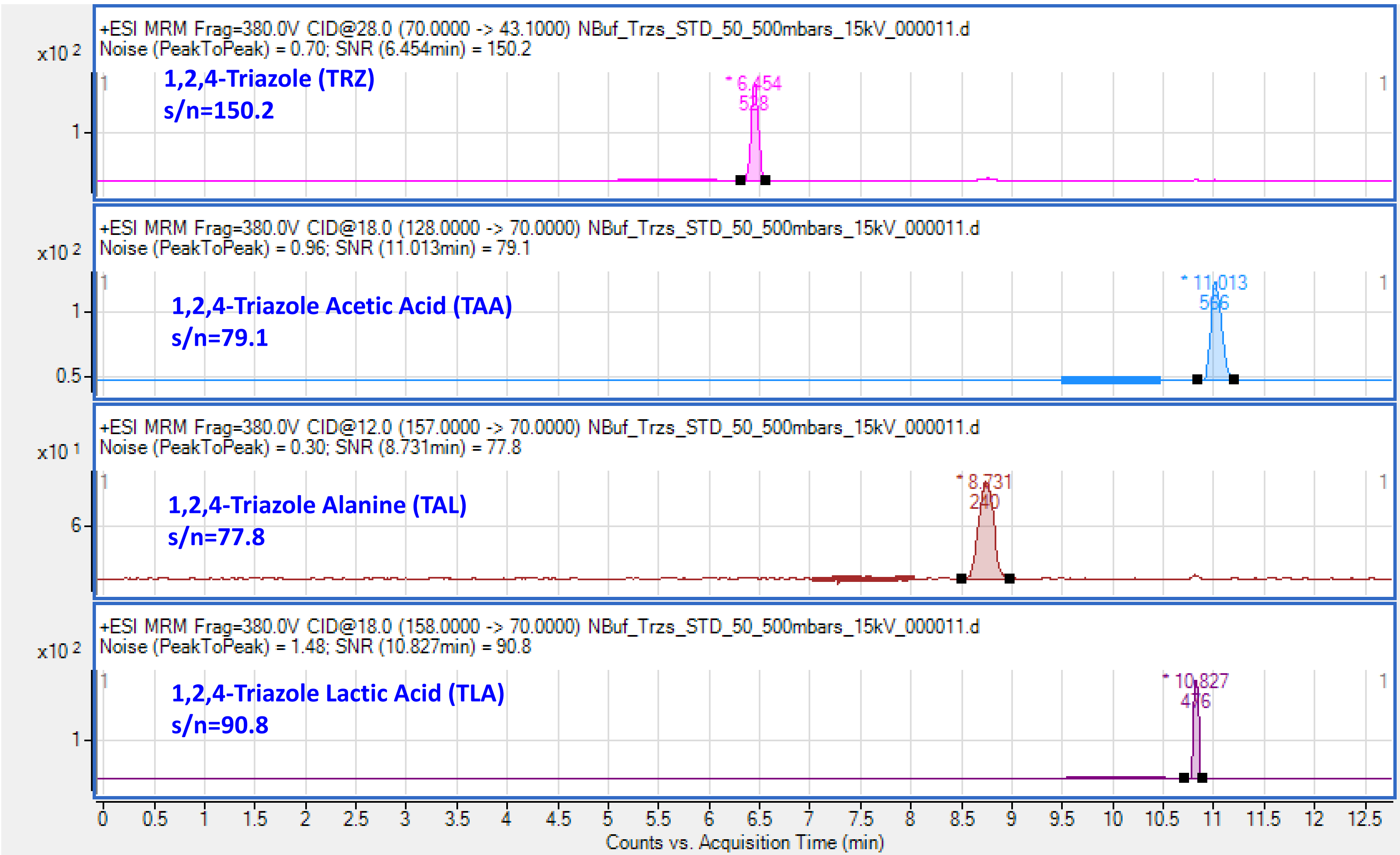
Drying Gas Temperature:	150 °C
Drying Gas Flow:	11 L/min
Nebulizer Pressure:	10 psi
Sheath Gas Temperature:	195 °C
Sheath Gas Flow:	3.5 L/min
Capillary (P/N):	4000 V
Nozzle Voltage (P/N):	2000 V
Resolution:	MS1 – Wide, MS2 – Wide

CE conditions

Capillary:	60cm x 50um i.d.
Buffer:	100mM Formic Acid
Injection:	500 mbars
Run:	15kV (+10mbar)
Sheath Liquid:	MeOH/H2O (50:50 v/v) + 0.1% Formic Acid
Sheath Flow:	10 µl/min

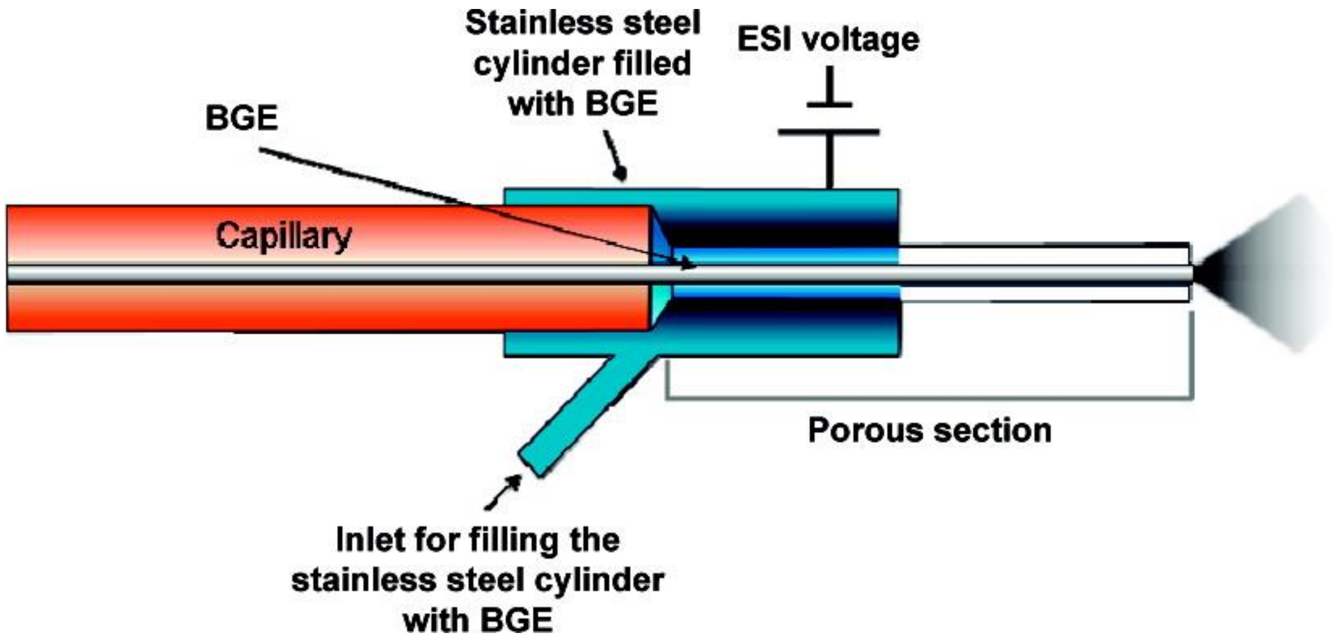
CE/QQQ Analysis of Triazole Metabolites

MRMs for the four triazole metabolites are shown here for a 50ppb standard.



New Developments in CE-MS "Sheathless" Interfacing

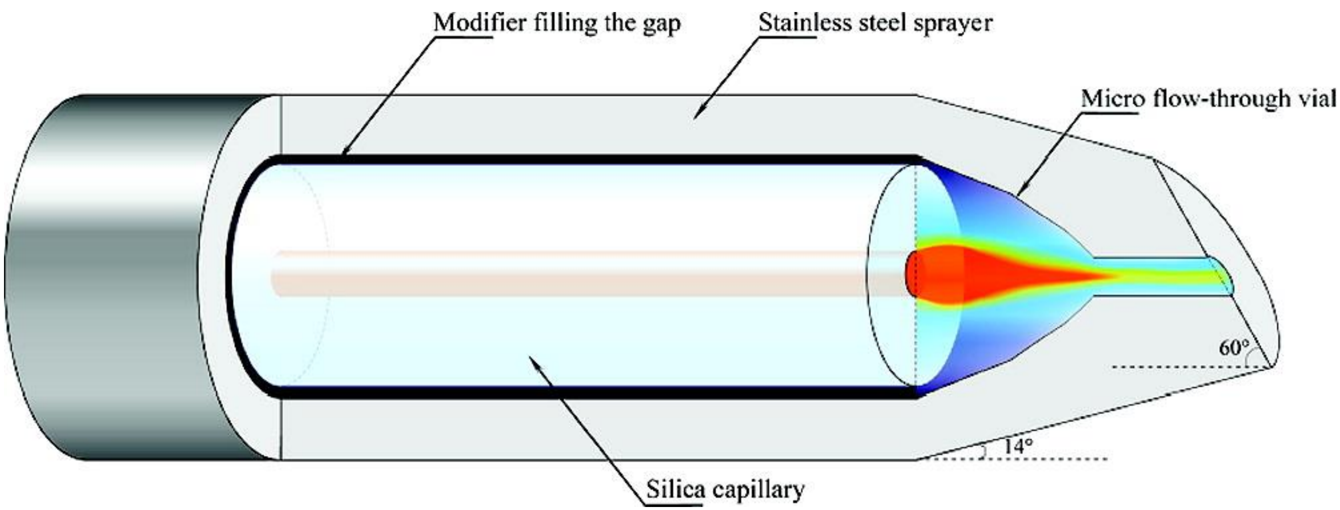
Porous tip approach, Mehdi Moini Anal. Chem., 2007, 79 (11), pp 4241–4246



Sensitivity sub-µM – nM
In absence of EOF, pressure assistance required to obtain a stable spray
Commercialized by Beckman-Coulter CESI 8000
Capillary OD/ID 220/30 µm

Figure published in: Jean-Marc Busnel; Bart Schoenmaker; Rawi Ramautar; Alegria Carrasco-Pancorbo; Chitra Ratnayake; Jerald S. Feitelson; Jeff D. Chapman; André M. Deelder; Oleg A. Mayboroda; *Anal. Chem.* **2010,** 82, 9476-9483. Copyright © 2010 American Chemical Society

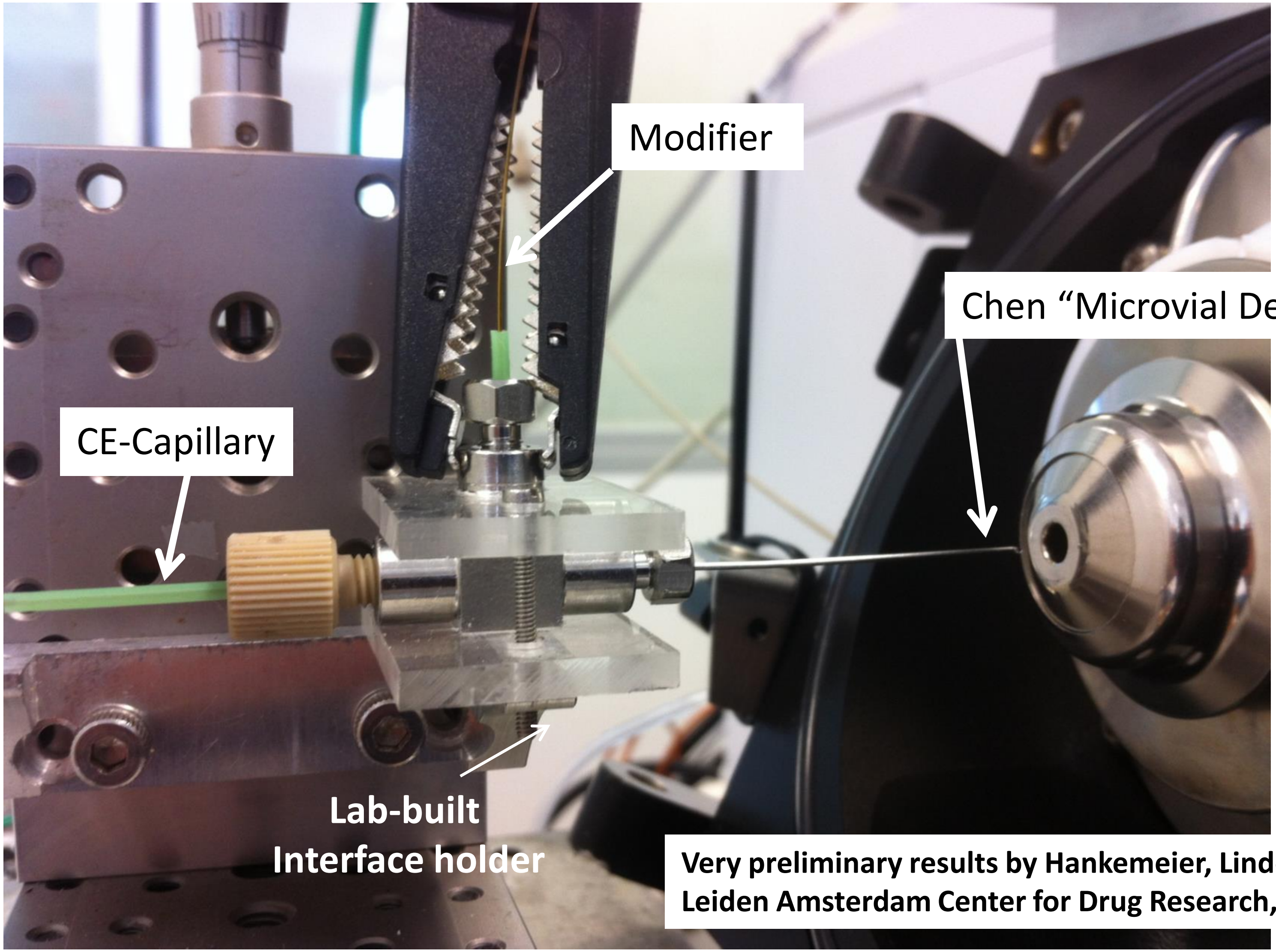
New Developments in CE-MS "Sheathless" Interfacing



Micro flow through vial – junction at the tip, Chen et al

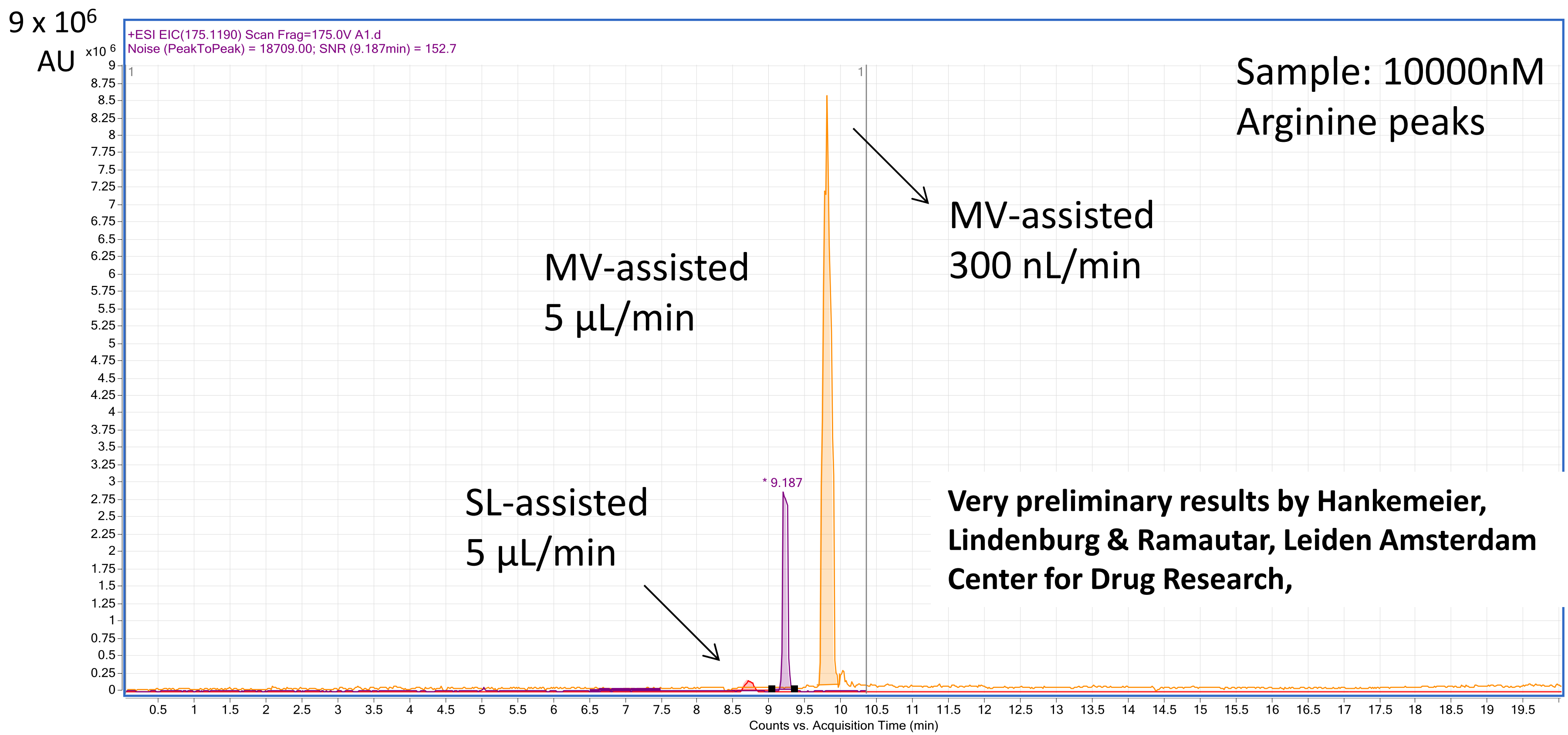
Figure published in: Xuefei Zhong; E. Jane Maxwell; David D.Y. Chen; *Anal. Chem.* **2011,** 83, 4916-4923.

CE-Agilent 6550 iFunnel-Q-TOF-MS via "Chen" Interface



Very preliminary results by Hankemeier, Lindenburg & Ramautar, Leiden Amsterdam Center for Drug Research,

Comparison between SL-assisted and MV-assisted: the signal



When MV is used, the signal increases dramatically…

Conclusions

- CE-MS with current sheath flow interface is a versatile and robust tool
- High flow LC-MS sources (Jet Stream) are compatible with ultra low flow CE separations
- Improved ion transport in MS results >10x higher sensitivity
- New “Sheathless” approaches result in higher sensitivity but practical usage needs to be proven

Acknowledgements

- Jean-Luc Veuthey, Julie Schappler, Isabelle Kohler and Serge Rudaz, University of Geneva, School of Pharmaceutical Sciences,
- Christian Wenz & Gordon Ross, Agilent Technologies, Waldbronn, Germany
- Thomas Hankemeier, Rawi Ramataur and Peter Lindenburg, Netherlands Metabolomics Center, Leyden University, Netherlands
- David Chen, University of British Columbia, Canada