A BRIEF REVIEW OF CE-MS ELECTROSPRAY INTERFACING: RETROSPECTIVE, CURRENT STATUS AND NEW DEVELOPMENTS

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Outline

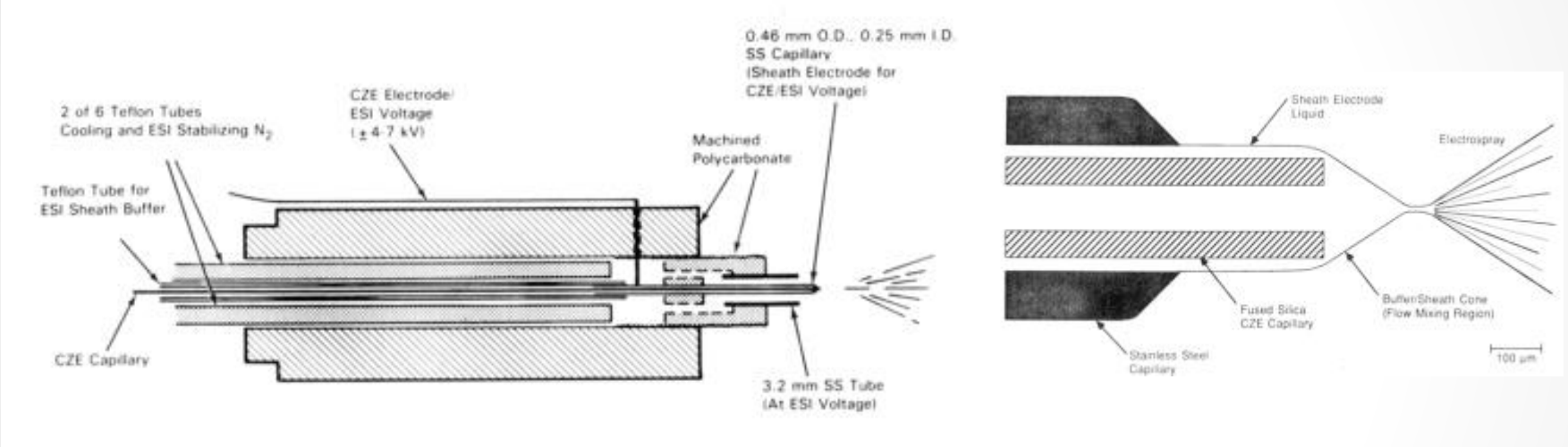
- A brief retrospective of coaxial sheath solvent flow interface for CE-ESI/MS coupling.
- Current status of CE-ESI/MS coupling by coaxial sheath solvent flow interface.
- New developments in CE-ESI/MS coupling; promises and reality.
- Is there a looming future of CE-MS?

Main challenges for CE-ESI/MS:

- No outlet vial/end electrode available when spraying into an MS
- How to apply the field between CE capillary exit and MS inlet or vice-versa to obtain an electrospray and at the same time maintain a field to drive the CE
- In CE, currents are typically 100-1000x larger than electrospray current; a safe electrical circuit and secure ground for handling the currents and fields
- In contrast with HPLC-ESI/MS, the solvent flow in CE, i.e. the EOF depends on its composition. This may impair the optimization of CE separation
- Like in HPLC; incompatibility of BGE's with non-volatile constituents and vacuum detection in MS. Eventually a BGE is selected that may or will compromise CE separation

CE-ESI/MS Coupling Retrospective

- 1988; Initial work with coaxial sheath solvent, R.D. Smith et al.*

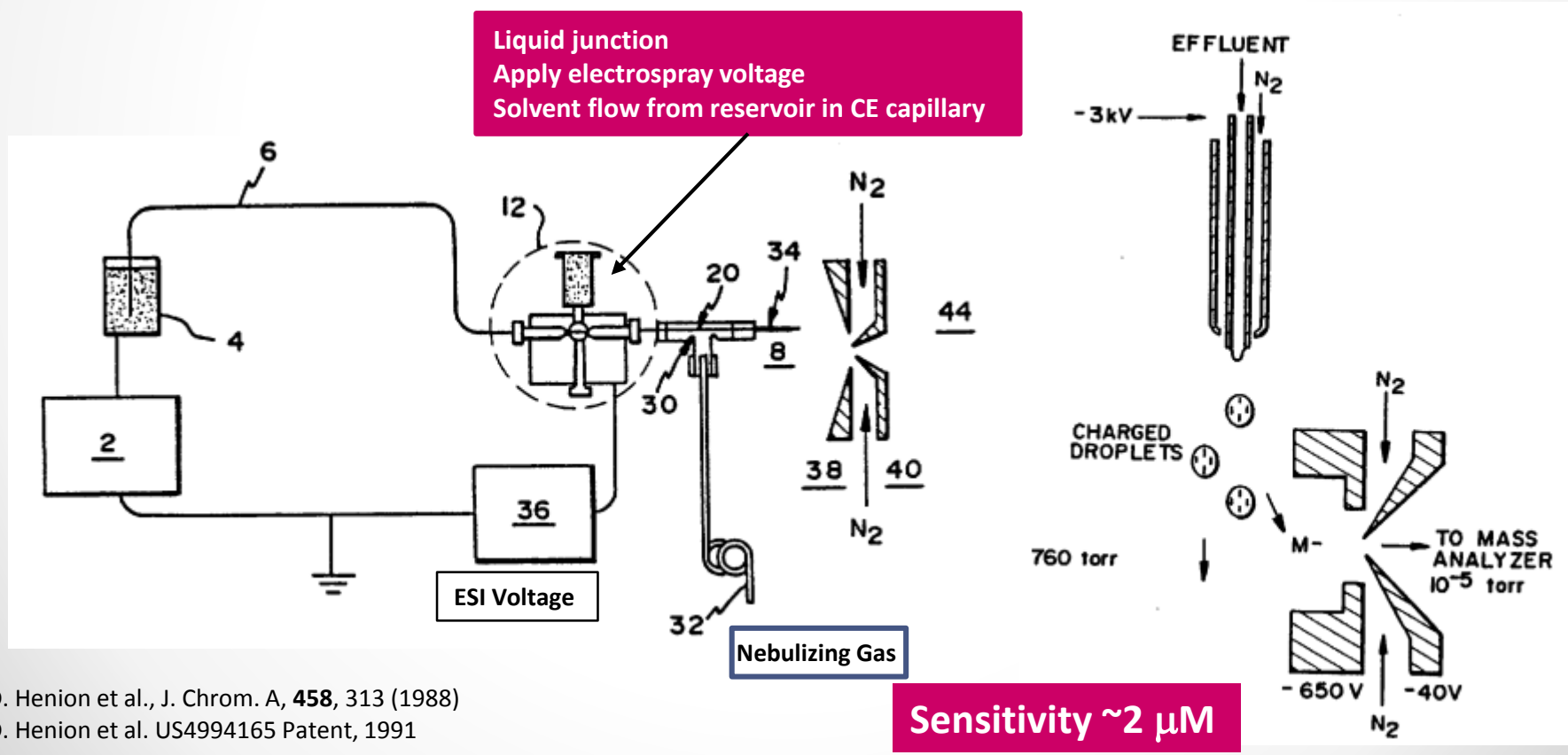


Sheath solvent delivered at 5-10 µL/min
Electrode in liquid electrical contact applying ES-voltage
Inert sheath gas to protect the spray
Stable and true electrospray

R. D. Smith et al, Anal. Chem. 60, 436, (1988)
R.D. Smith, C.J. Barinaga, H.R. Udseth, Anal. Chem., 60, 1948 (1988)
R.D. Smith, H.R. Udseth, Nature, 331, 639 (1988).

CE-ESI/MS Coupling Retrospective

- 1988; Initial work with coaxial sheath solvent, R.D. Smith et al.
- 1988; Ion spray approach with liquid junction, J.D. Henion et al.*



J.D. Henion et al., J. Chrom. A, **458**, 313 (1988)
J.D. Henion et al. US4994165 Patent, 1991

Sensitivity ~2 µM

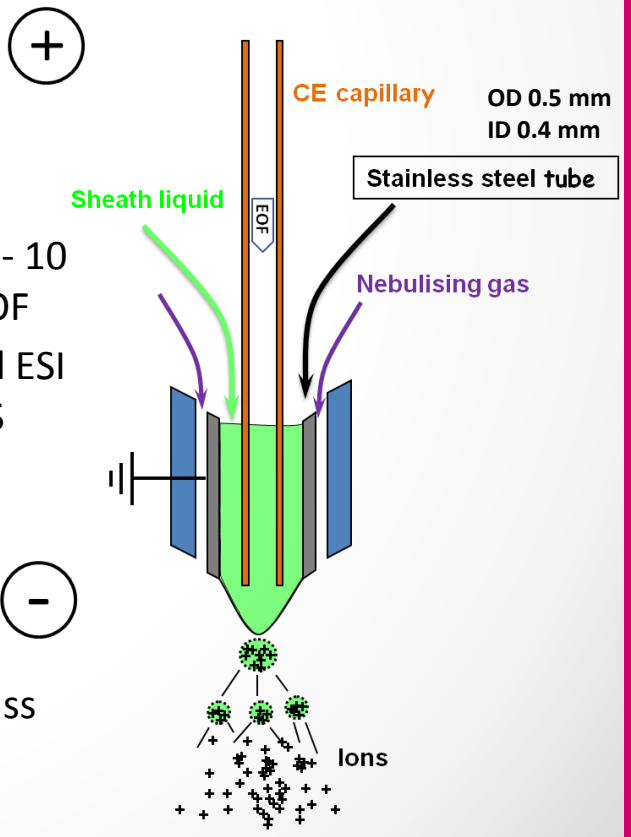
CE-ESI/MS Coupling Retrospective

Since 1995:

- In practice, skilled users had to resort to in-house adaption of commercial (nano)LC-MS sprayers to do CE-ESI/MS
- Hewlett-Packard (Agilent Technologies) introduced Triple Tube Sprayer (co-used by Bruker) with an integrated CE-MS system

Characteristics of "Triple Tube" Sprayer Interface

- **Sheath solvent** is added to the CE effluent at a rate of typically 1 - 10 µL/min. Spray becomes independent of BGE composition and EOF
- **Spray needle** (gray) is grounded. Common return path for CE and ESI current. Bubbles are transported out. ESI voltage provided by MS
- **Nebulizing gas** to assist spray formation
- **Sheath solvent composition** dominates electrospray ionization chemistry
- **Compliant with different ionization modes**: ESI, APCI, APPI
- **Orthogonal configuration** (LC-MS) lets neutrals & big droplets pass

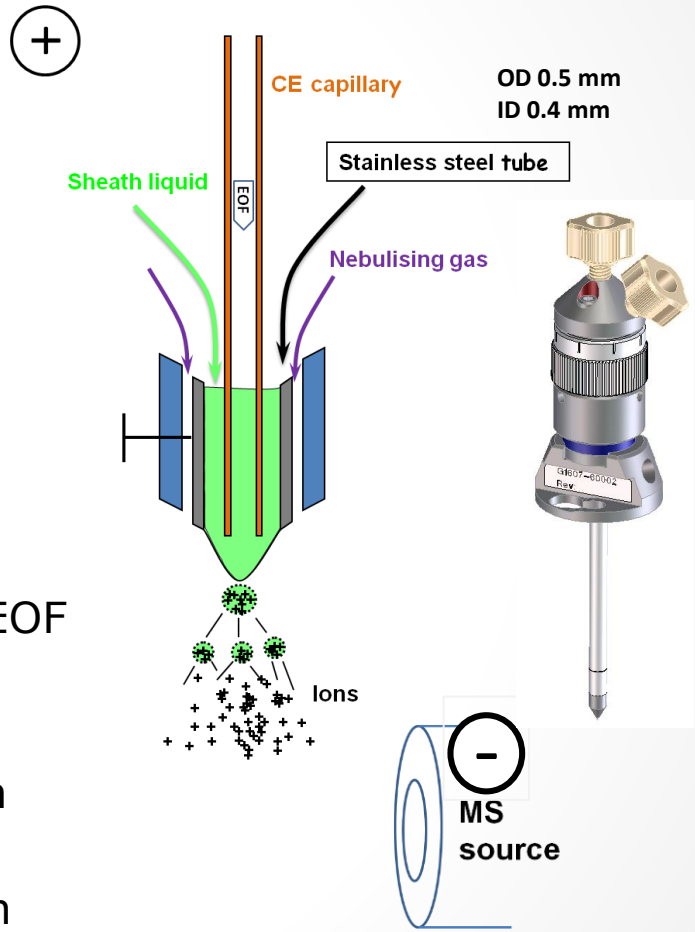


CE-ESI/MS - Current Status

Agilent Triple Tube Sprayer IF

- ☺ Since 1995 only complete commercial system for CE-ESI/MS
- ☺ Proven robustness and reliability
- ☺ Typical sensitivity 0.5 - 10 µM (in sample concentration)

But ....



Sensitivity becomes compromised

- ☹ Concentration sensitive detection!
- ☹ Solute concentration is reduced 5 - 50x by the sheath solvent depending on the actual EOF
- ☹ Because of the higher flow rate no nano-electrospray (<100 nL/min)

Pneumatic assistance required to establish the spray

- ☹ Undesirable hydraulic flow is observed, which need counter measures

Galvanic reactions on the sprayer needle

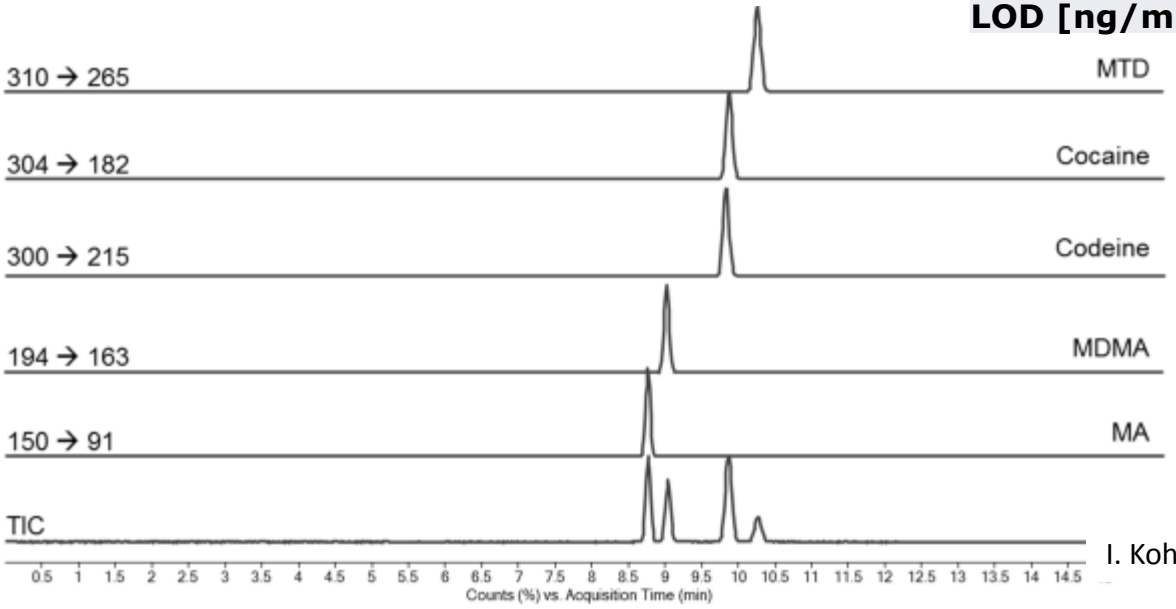
Current Status of CE-ESI/MS Coupling

Agilent Triple Tube Sprayer IF

Improvements from Agilent Technology

- Optimized sprayer geometry/Pt needle avoiding corrosion
- Allows to use LC-MS Jetstream IF technology for CE-MS
- Higher ion capture with (Agilent 6x90 MS series)
  - Hexabore inlet capillary
  - Ion funnel

Aqueous Standard Drugs of Abuse



	MA	MDMA	Codeine	COC	MTD
LOD [ng/mL]	**0.5**	**0.5**	**5**	**2**	**50**

Sensitivity: ~ 2 - 200 nM concentration in standard

I. Kohler et al., Analytica Chimica Acta, 780 (2013) 101

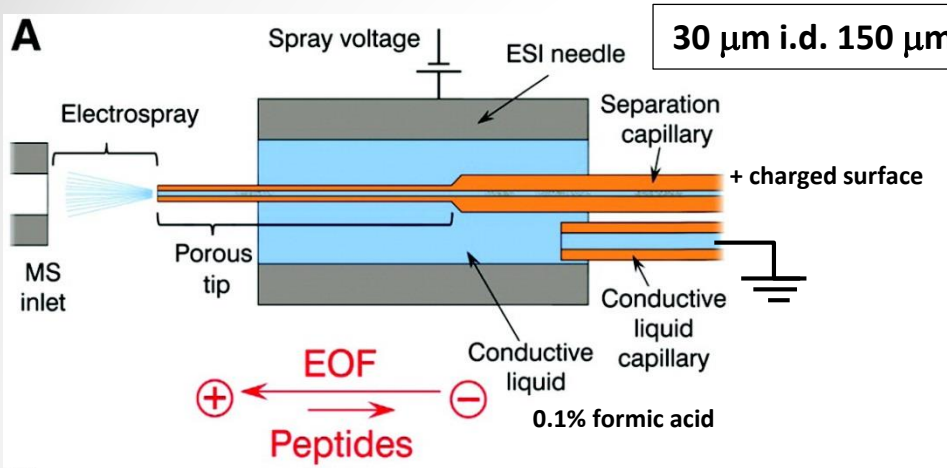
Recent Developments in CE-MS Coupling

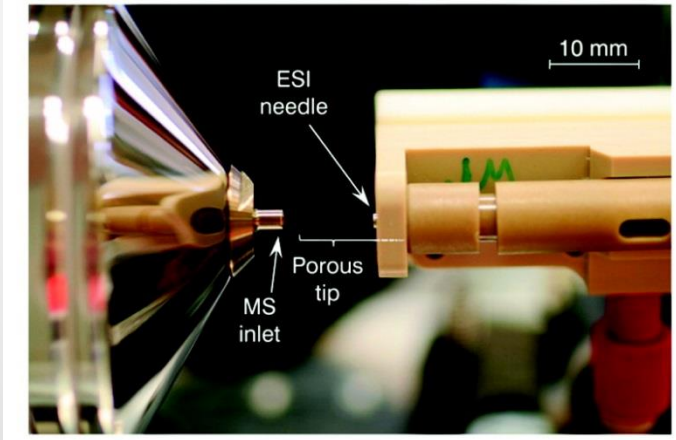
- Porous tip approach*
- Micro flow-through vial**
- EOF driven sprayer***
- Sheath liquid contact approach****

*M. Moini, Anal. Chem., **79**, 4241 (2007)
D.D.Y. Chen et al., Anal. Chem. **83, 4916 (2011)
***N. Dovichi et al., Rapid Comm. Mass Spec., **24**, 2554 (2010)
****R.D. Smith et al., Anal. Chem., **84**, 10395 (2012)

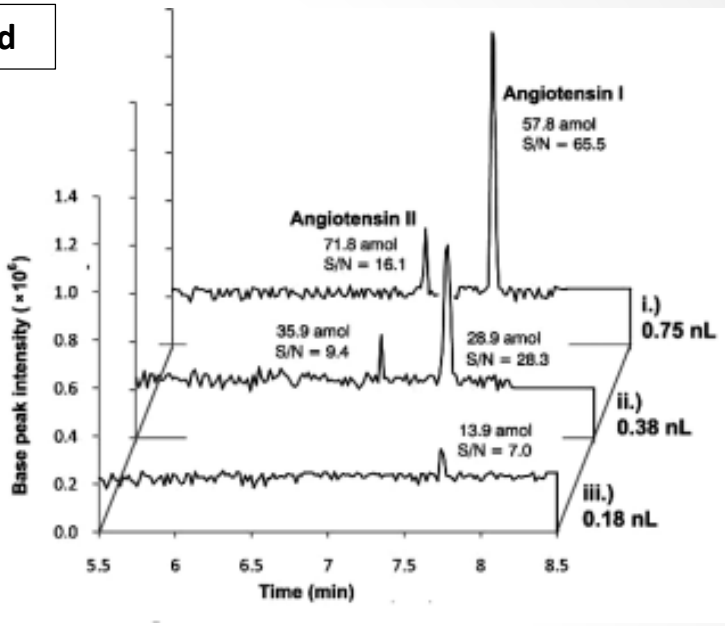
Recent Developments in CE-MS Coupling

Porous Tip Approach





The high-sensitivity porous sprayer interface (A) schematic and (B) photograph of the prototype interface.

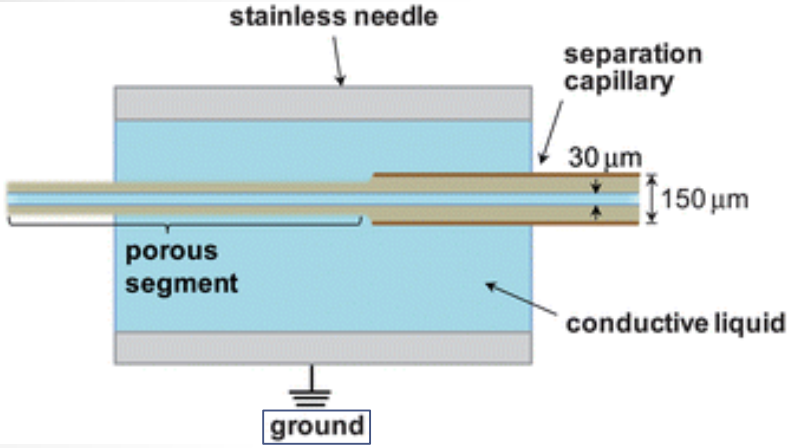


- Sensitivity: 10-20 nM AT1 concentration in sample
- >200 successive runs (pers. comm.)

Figures taken from:
H. Lindner et al., Anal. Chem., **83**, 7297 (2011)

Recent Developments in CE-MS Coupling

Comparison Coaxial Sheath Flow and Porous Tip (T. Soga et al.)



vs.

System: Agilent 6220 Accurate-Mass TOF LC/MS with Beckman Coulter PA800 plus CE and Agilent G7100 series CE respectively

Sample: Cationic metabolites, 2.3 and 2.6 nL injected resp.

BGE & Contact: 1 M formic acid

BGE & Sheath: 1 M formic acid, MeOH/Water with 0.1% hexakis

Capillaries: 50 µm i.d. and 30 µm i.d. resp.

Sensitivity: 10-100 nM concentration in sample with porous tip

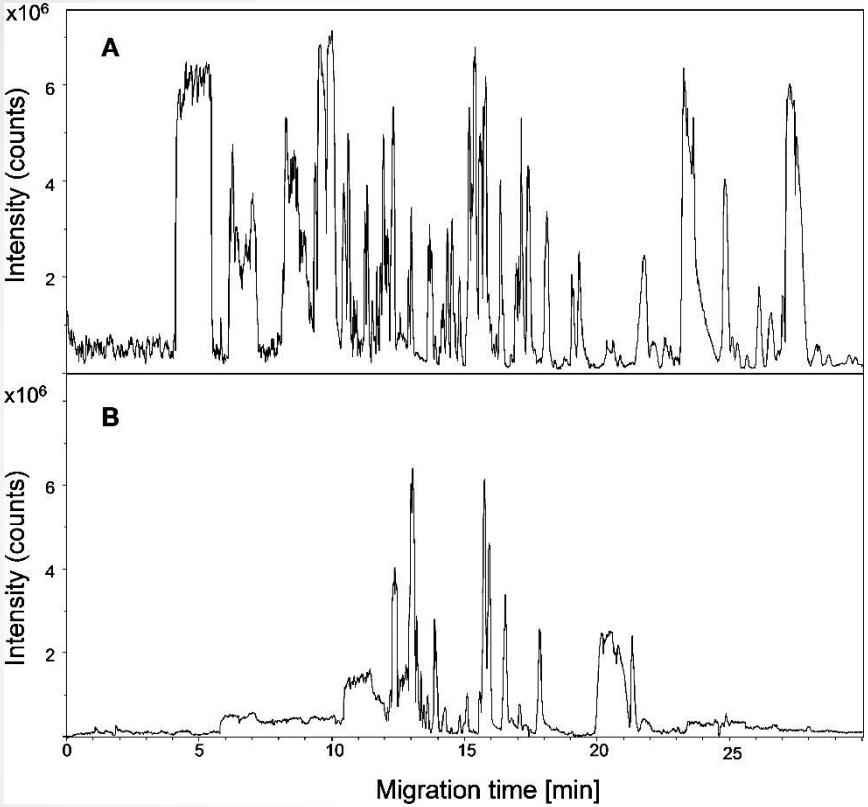
Relative: 0.2 – 20x sheathless/coaxial sheath flow

Robustness: 180 successive runs

T. Soga et al., Analyst, **137**, 5026 (2012)

Recent Developments in CE-MS Coupling

Comparison Coaxial Sheath Flow and Porous Tip (Ramautar et al.)



CE :	A: bare, 40 μm x 150 μm
	B: bare, 50 μm x 365 μm
BGE & Contact:	A: 10% HOAc, pH 2.2
BGE & Sheath:	B: Same, 0.1% HOAc/ Methanol 50/50, 4 μL/min
MS:	Bruker ToF → Tip grounded

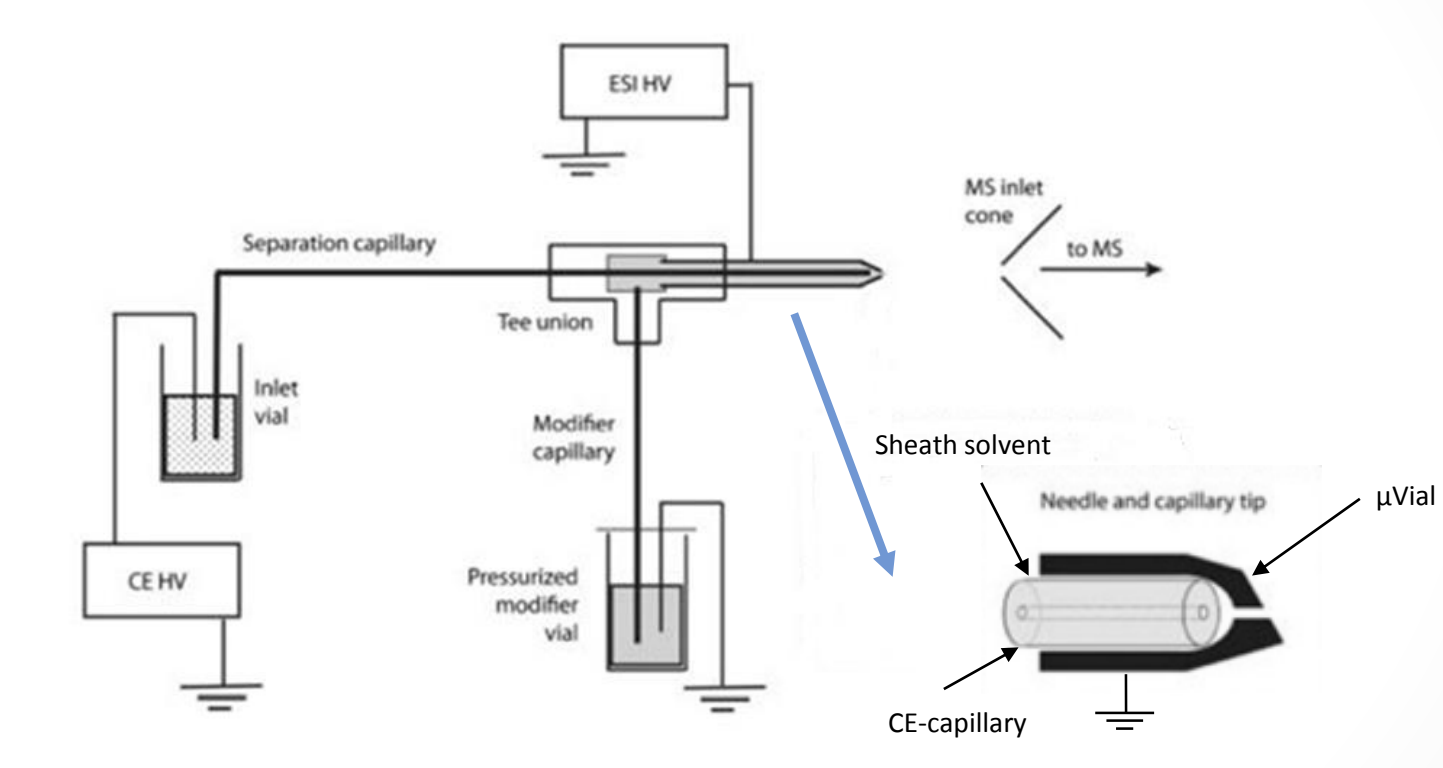
A. Base peak electropherogram (*m/z* 50–450) of human urine obtained with sheathless CE-MS using a porous tip sprayer. LOD 10-100 nM

B. Base peak electropherogram (*m/z* 50–450) of human urine obtained with CE-MS using a sheath-liquid interface. LOD 300-1000 nM

R. Ramautar et al., Anal. Chem., **84**, 885 (2012)

Recent Developments in CE-MS Coupling

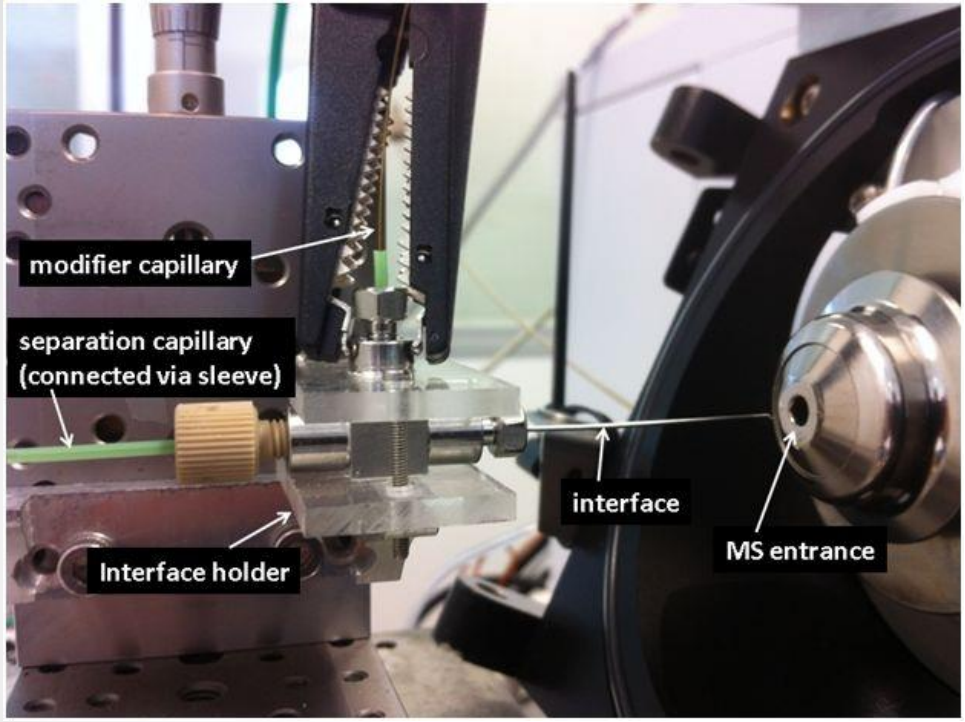
Micro Flow-Through Vial (D.D.Y. Chen et al.*)



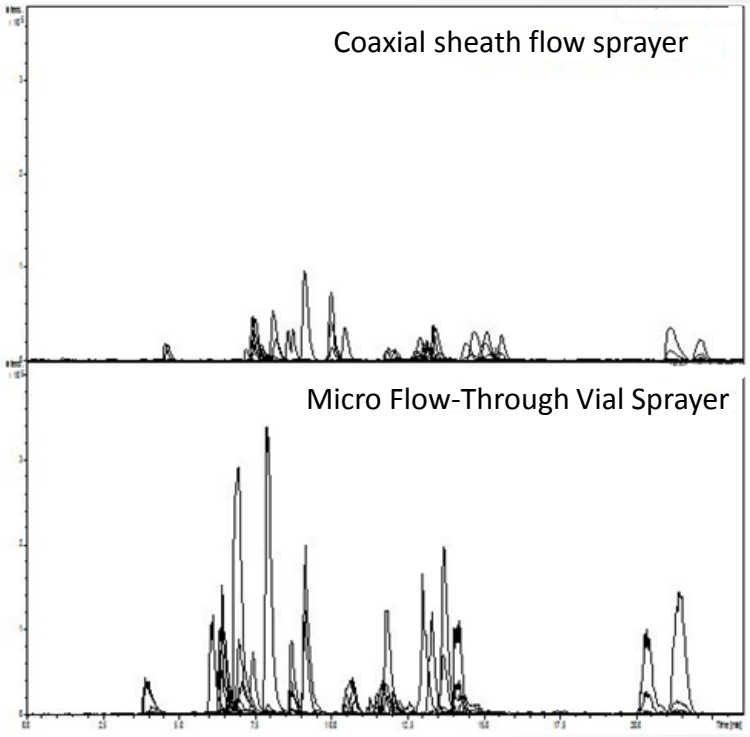
*D.D.Y. Chen et al. Anal. Chem. **83**, 4916 (2011)

Recent Developments in CE-MS Coupling

Micro Flow-Through Vial Practical Setup*



Preliminary results

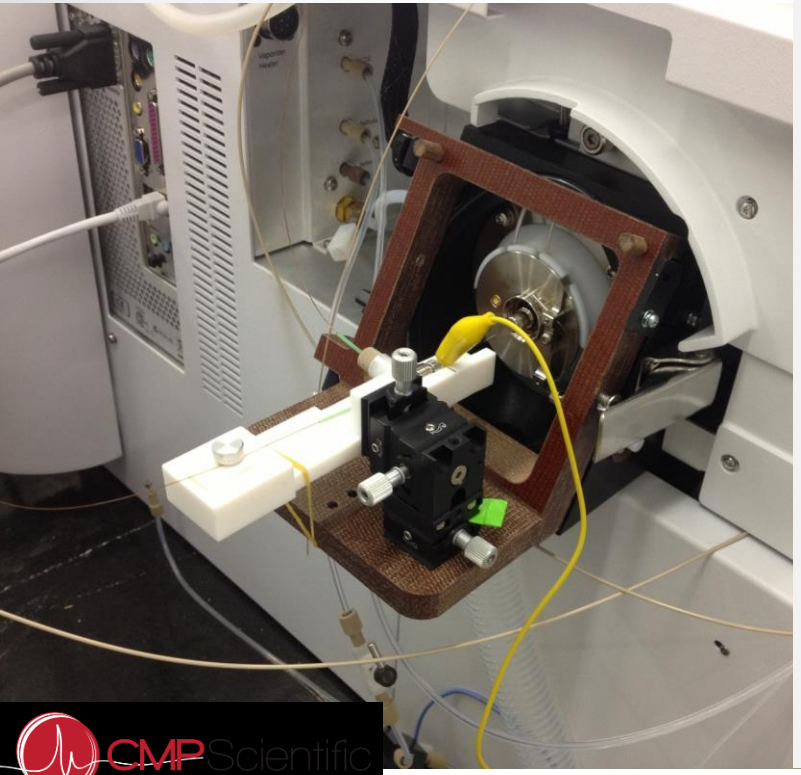


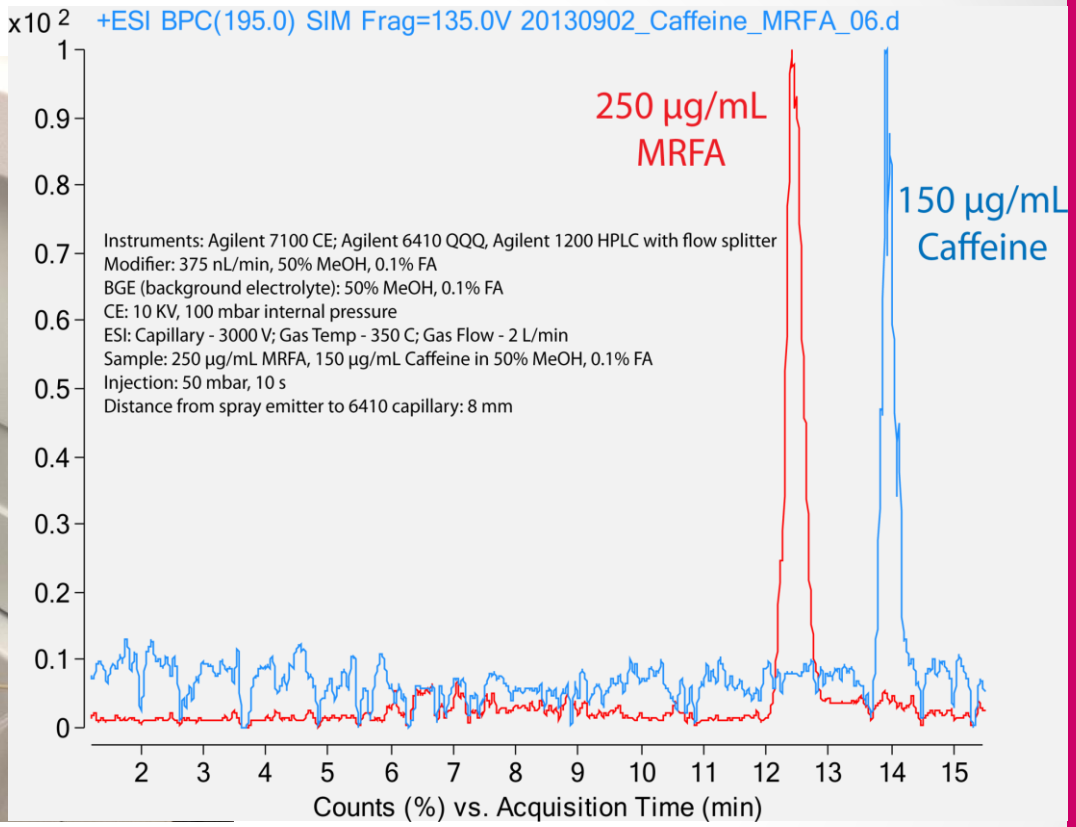
Sample: Cationic Metabolites from Human Metabolome Technologies
Sensitivity: 0.2 -4 μM
Improvement: 0.2 – 20x

*Results and Photo courtesy of Peter Lindenburg et al., Netherlands Metabolomics Center, Leiden, The Netherlands

Recent Developments in CE-MS Coupling

Micro Flow-Through Vial Common Ground*



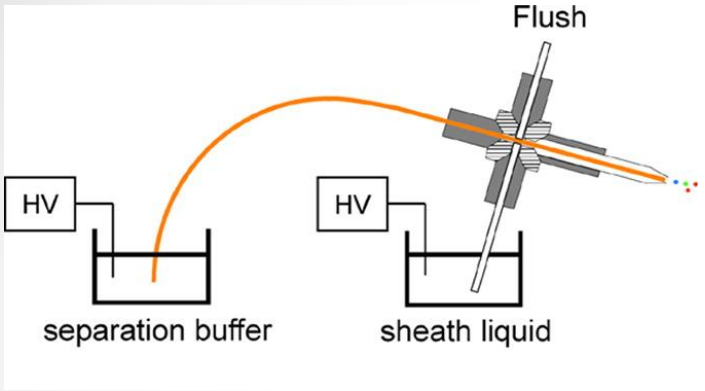


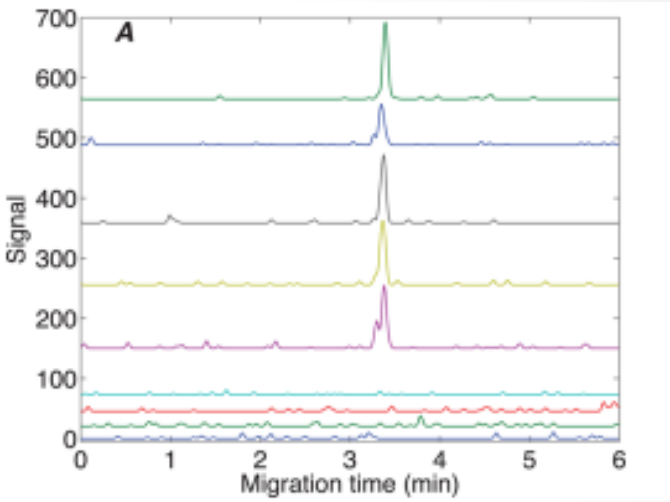
Sample: calibration mixture for electrospray ion sources (caffeine, MRFA (met-arg-phe-ala))
Sensitivity: approx. 5 µM

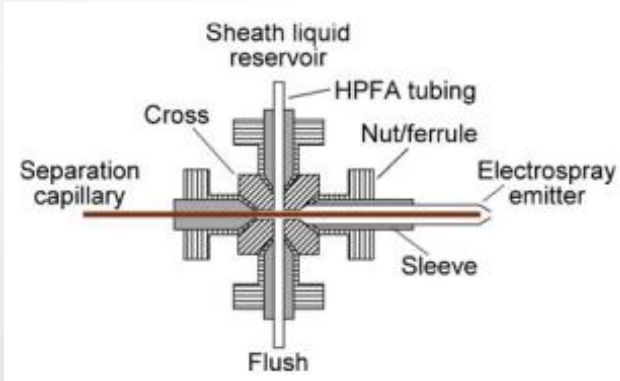
*Results and Photo courtesy of David Chen and CMP Scientific

Recent Developments in CE-MS Coupling

EOF Driven Sprayer (N. Dovichi et al)







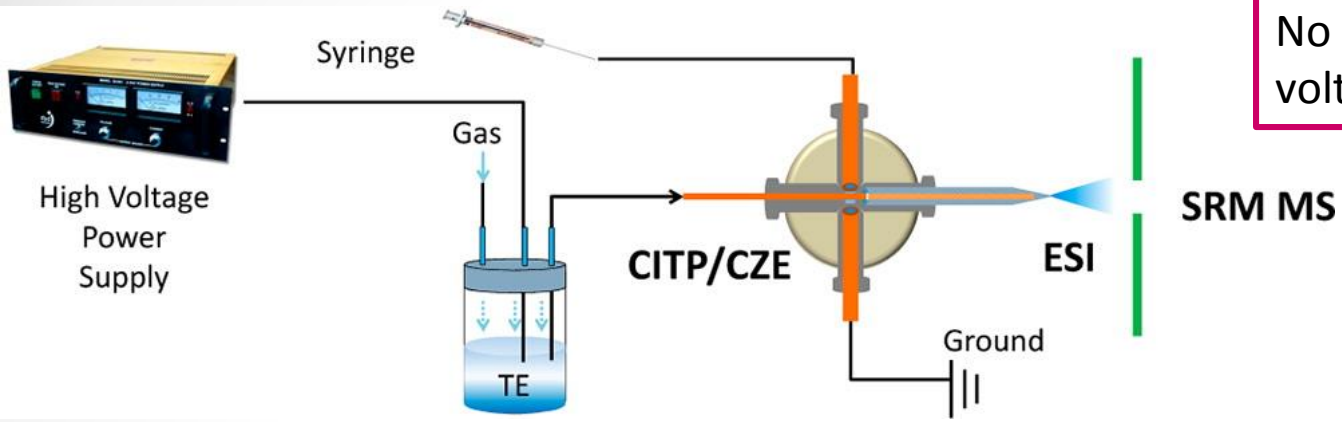
FS separation capillary 50x150 µm
Borosilicate emitter capillary 0.75x1 mm, orifice 5 µm
BGE 10 mM ammonium acetate, pH 5.5
Sheath solvent MeOH/0.1% formic acid
Sample: short peptides

Sensitivity: < 1 nM in sample concentration

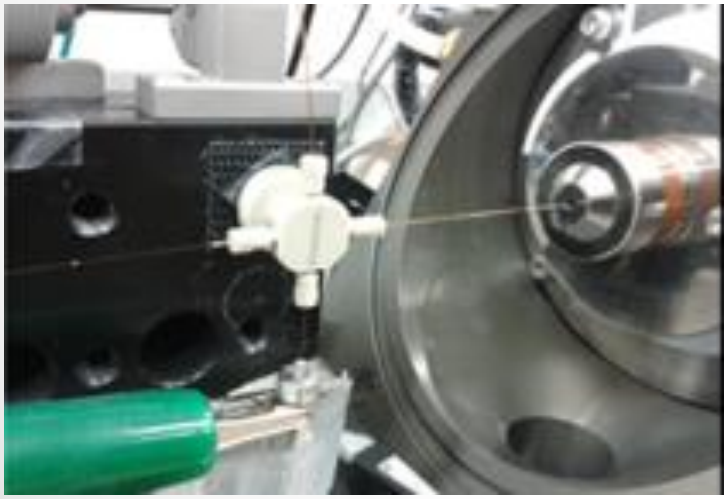
N. Dovichi et al., Rapid Comm. Mass Spec., 24, 2554 (2010)

Recent Developments in CE-MS Coupling

Sheath liquid contact approach (R.D. Smith et al.)



No additional ESI voltage used.



Separation capillary: FS 75x150 µm
Emitter capillary: FS 200x350 µm, end etched with HF and orifice 50 µm
BGE: 25 mM ammonium acetate, pH 4
Sheath solvent and TE:9/1 0.1 M acetic acid/methanol

Sample: short peptides in BSA digest

Sensitivity: 50 pM with CITP sample pre concentration

*R.D. Smith et al., Anal. Chem., **84**, 10395 (2012) and Chenchen Wang et al, Poster presented at MSB2013, Charlottesville

Rel. Assessment CE-MS Coupling

	Triple Tube	Porous Tip	Flow-through µVial	EOF Driven Tip	Smith approach
Sensitivity (LOD)	0.5 µM[b]/20 nM[a]	20 nM[b]	0.2 – 5 µM[c]	1 nM	50 pM[d]
Robustness/Reliability	xxx	xx	xx[c]	?	?
Ease of Use	xxx	xx	xx	?	?
Standard Capillaries?	YES	NO[e]	YES	NO[e]	NO[e]

a. achievable with best MS equipment
b. See table 1 in, R. Ramautar et al., Anal. Chem., **84**, 885 (2012) and T. Soga et al., Analyst, **137**, 5026 (2012)
c. improvements needed and possible
d. In combination with cITP
e. special capillaries (I.D., emitter tip), wall coating for reliable EOF needed

Is there a looming future for CE-MS?

- Achieving highest sensitivity remains top objective; but…
  - unlike HPLC, CE has limited sample volume loading capacity and cannot be scaled like HPLC.
  - in contrast to HPLC with SPE pre-concentration, sweeping or cITP pre-concentration methods are regarded "difficult".
- Fact is ….
  - given the same amount entered into the MS, CE results in higher peaks than HPLC!
  - the premier user's interest though is the analyte concentration in the sample
  - therefore, CE-MS will be preferred for measurement of polar/charged analytes in very small sample volume
- Conventional coaxial solvent sheath flow IF pairs adequate sensitivity (with up-to-date MS) with ease of use and robustness
- Porous tip and μVial-flow through IF seem a promising pathway towards CE-ESI/MS.
- Commercialization (affordable) will be the key for success of new sheathless CE-ESI/MS coupling methods

Is there a revitalization of CE?

Quote Peter Schoenmakers…

- Indeed the # of papers on CE may be declining (J. Chromatography, Electrophoresis) but the revitalization of CE is in CE-MS, which papers are not published in JCHROM or EP but in journals on proteomics, metabolomics, glycomics, foodomics biopharmaceutical analysis etc.

Acknowledgements

- Paul Goodley, Alex Mordehai, Hans-Peter Zimmermann Martin Greiner with Agilent Technologies for providing insights in the development of the triple tube IF
- Isabelle Kohler, Julie Schappler, Serge Rudaz and Jean-Luc Veuthey of Pharmaceutical Sciences at University of Geneva
- Herbert Lindner, Innsbruck Medical University
- Peter Lindenburg, Rawi Ramautar, Thomas Hankemeier Netherlands Metabolomics Center, Leiden University
- David Chen, University of British Columbia, Canada
- CMP Scientific, Hoboken, USA

A PDF-copy of this presentation can be found at http://www.rozing.com (registration required!)