Effective coupling of CE with nanoESI MS via a true sheathless metal-coated emitter interface for robust and high sensitivity sample quantification

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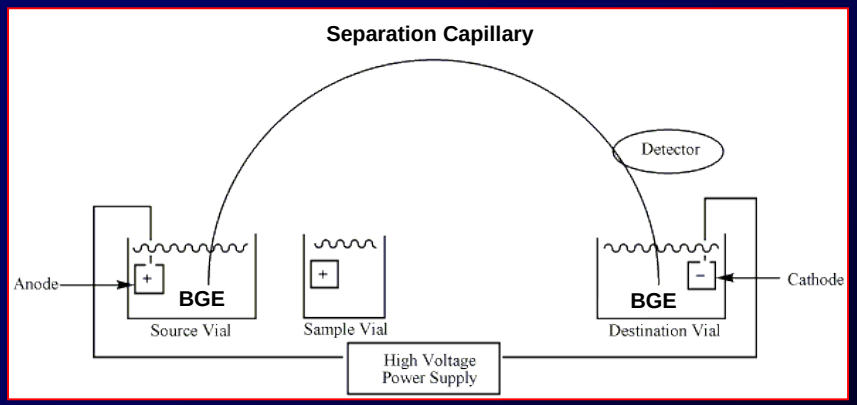
Characteristics of capillary electrophoresis mass spectrometry (CE-MS)

- High separation efficiency.
- Broad analyte coverage including structure variants.
- Sensitive detection (an intrinsic low flow separation technique).
- Easy to set up.

Limitations

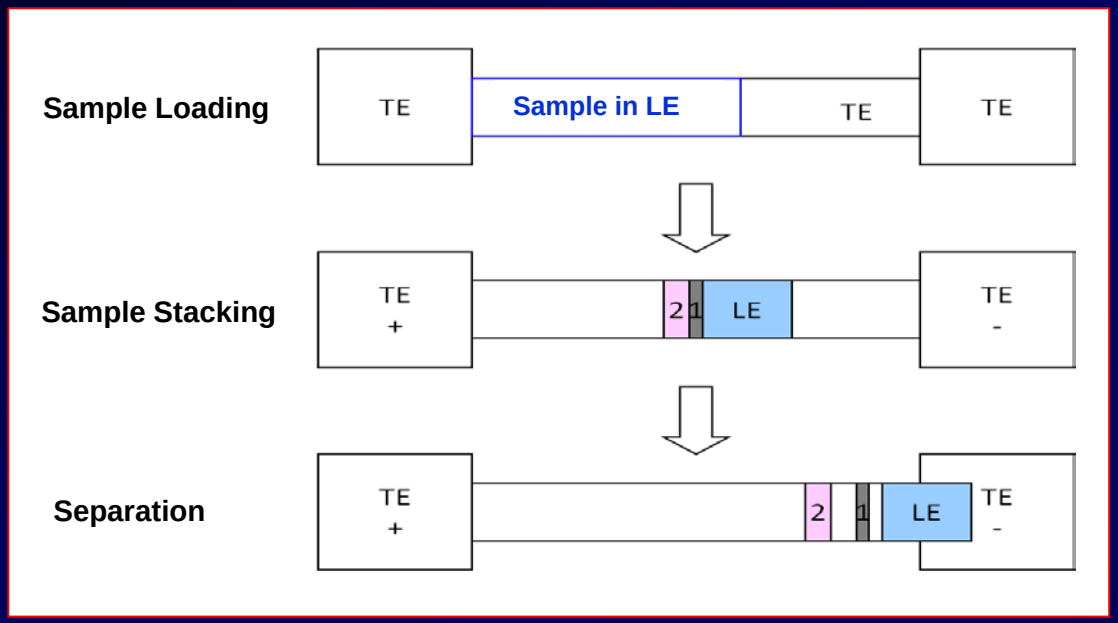
- Small sample loading capacity.
- Lack of a robust CE-MS interface.



CE or CZE separation, Background electrolyte (BGE): 9:1 volume ratio of 0.1 M acetic acid in deionized water to methanol.

A effective solution to limited sample loading capacity

Transient capillary isotachophoresis (CITP/CZE):



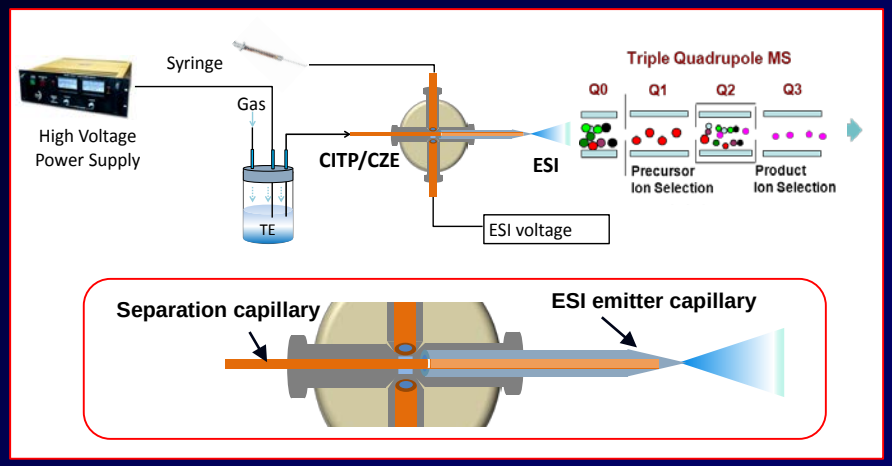
Trailing electrolyte (TE): 9:1 volume ratio of 0.1 M acetic acid in deionized water to methanol.

Leading electrolyte (LE): 25 mM ammonium acetate in deionized water with the solution pH adjusted by adding acetic acid to pH = 4.

- Combined sample stacking (focusing) and separation to allow a sample loading volume up to 1/3 of the total separation capillary volume (~ 2 to 3 uL).
- Selective enrichment of low abundance species in a complex mixture.
- Mobility dependent focusing: $C_A = C_L / \{[\mu_L / (\mu_L + \mu_R)]\,[(\mu_A + \mu_R) / \mu_A]\}$.

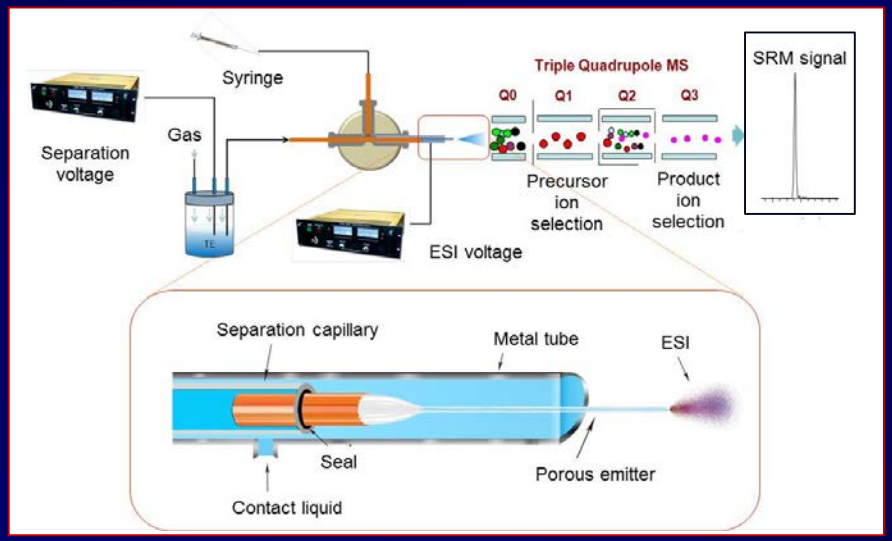
Two previous interfaces for online CITP/CZE-SRM MS

a) Sheath liquid interface*:



- Electrical contact for ESI through the liquid junction at the emitter tip.
- ESI voltage at the waste line to avoid gas bubbles for stable electrospray operation.
- Separation capillary: 75 µm i.d., 150 µm i.d., 85 cm long, 4 µl total volume; laser pulled ESI emitter capillary: 200 µm i.d., 360 µm o.d., ~50 µm tip.
- Limit of quantitation: 50 attomoles (at ~100 nL/min ESI flow rate).

b) Sheathless interface**:



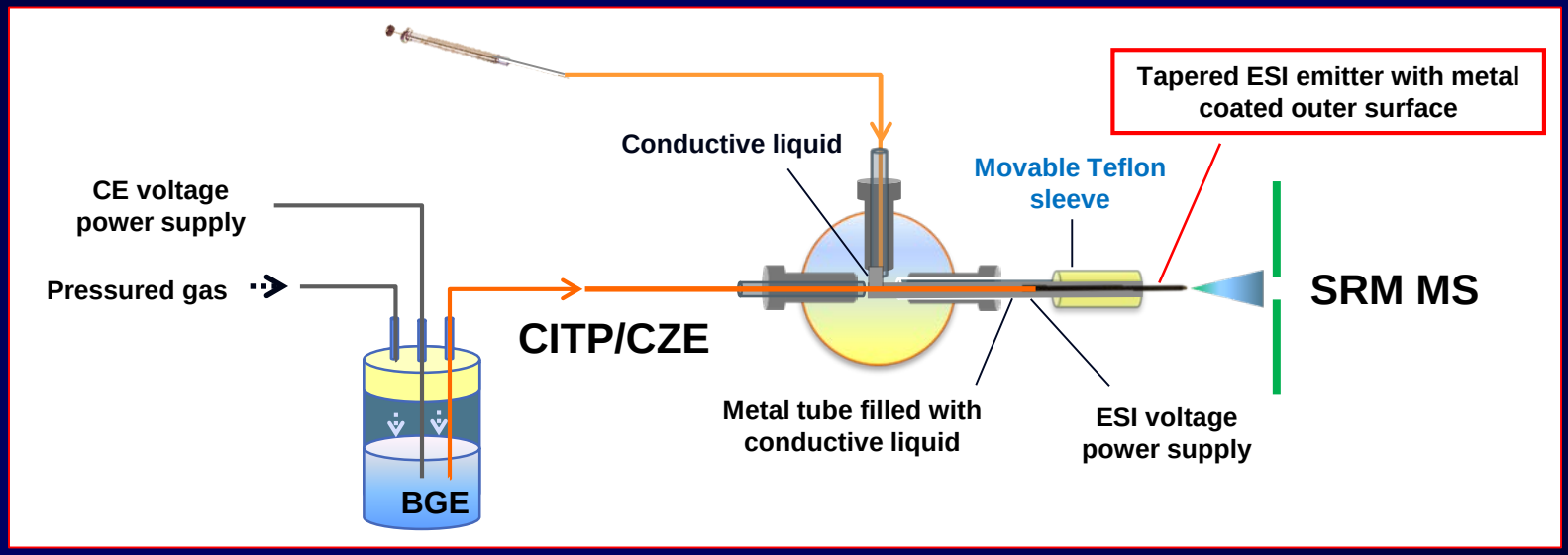
- Electric contact for ESI through conductive liquid enclosed in a short metal tube and porous wall of the emitter capillary.
- Separation capillary: 100 µm i.d., 360 µm i.d., 95 cm long, 7.5 µl total volume for large sample loading; etched ESI emitter capillary: 20 µm i.d., 90 µm o.d. for stable NanoESI.
- No sample dilution and easy exchange of emitter capillary.
- Limit of quantitation: 25 attomoles (at 60 nL/min ESI flow rate).

* C. Wang et al., *Anal. Chem.*, **84,** 10395-10403 (2012); ** C. Wang et al., *Anal. Chem.*, **85,** 7308−7315 (2013).

Weakness of the interfaces:

- Require careful adjustment and control of fabrication process to achieve reproducible and sensitive sample analysis.
- Difficult to operate for an inexperienced user.

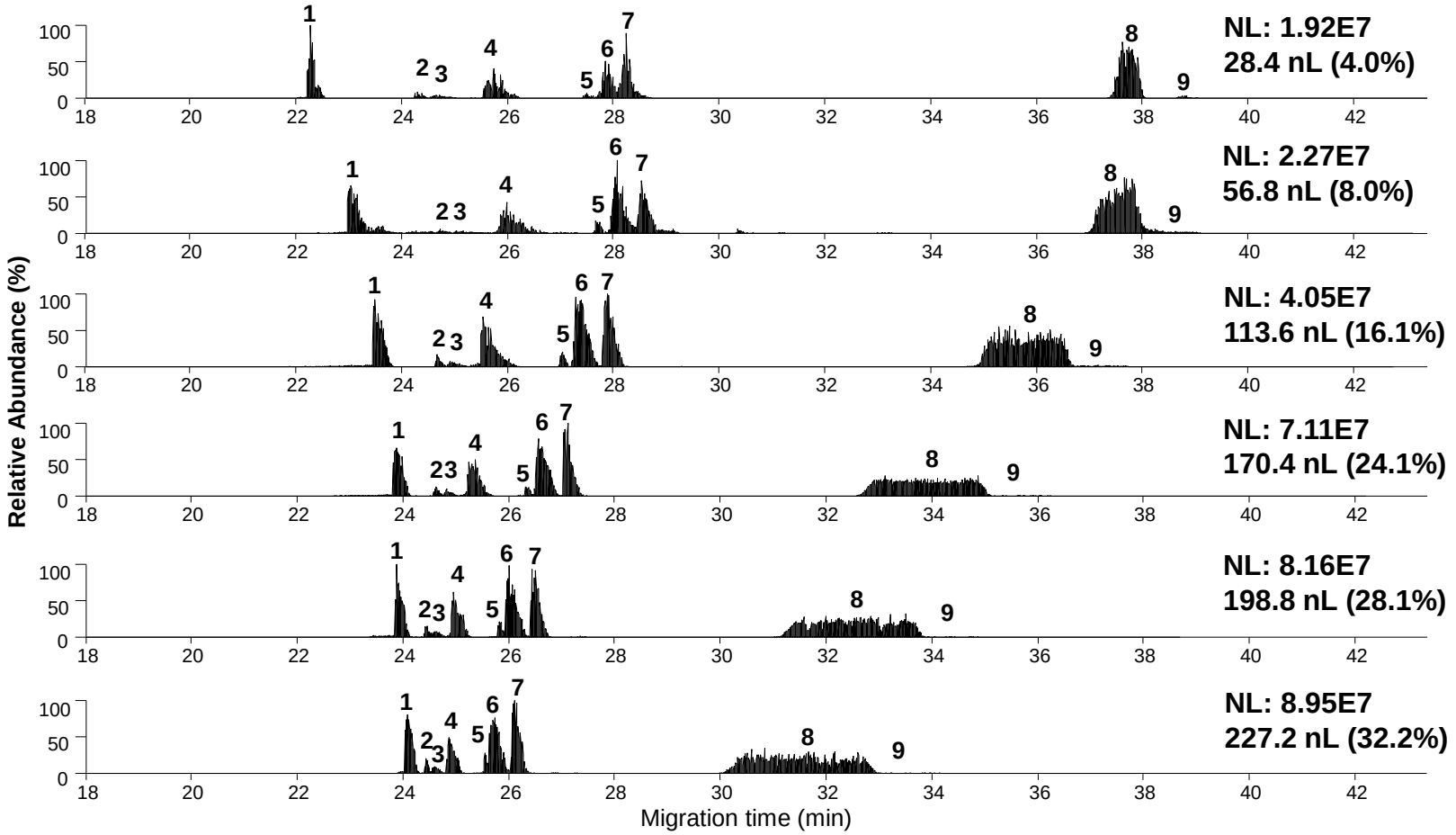
Development of a robust sheathless CE-MS interface*



- A commercial CE capillary (360 μm o.d., 30 μm i.d.; 100 cm long) with an integrated metal coated ESI emitter having a constant i.d. and tapered o.d..
- Electric contact for ESI through conductive liquid enclosed in a short metal tube and the metal coated outer surface of the emitter.

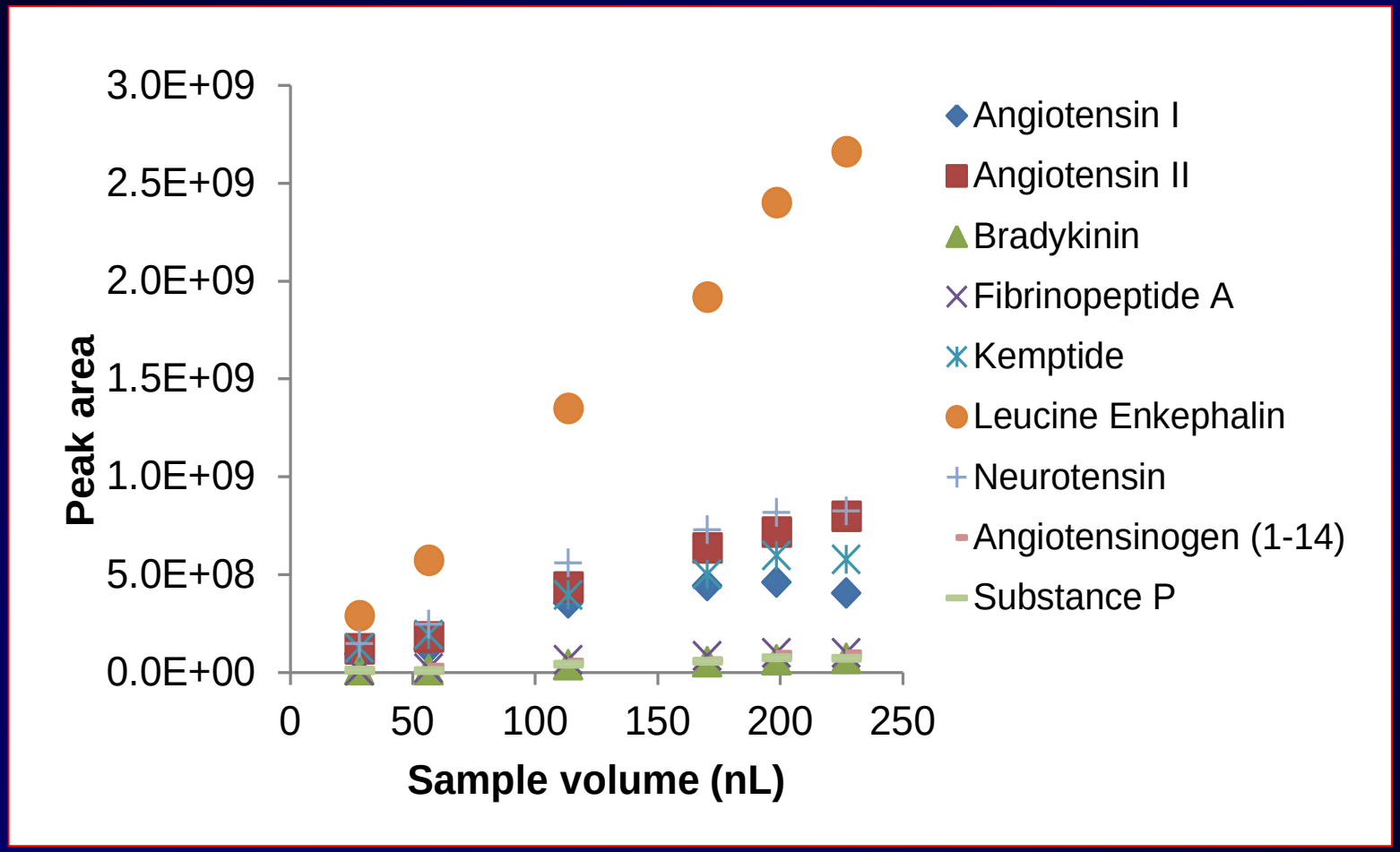
* X. Guo et al., *Anal. Chem.*, **88,** 4418−4425 (2016).

Effect of sample injection volume on CITP/CZE-MS with the new sheathless CITP/CZE-MS interface



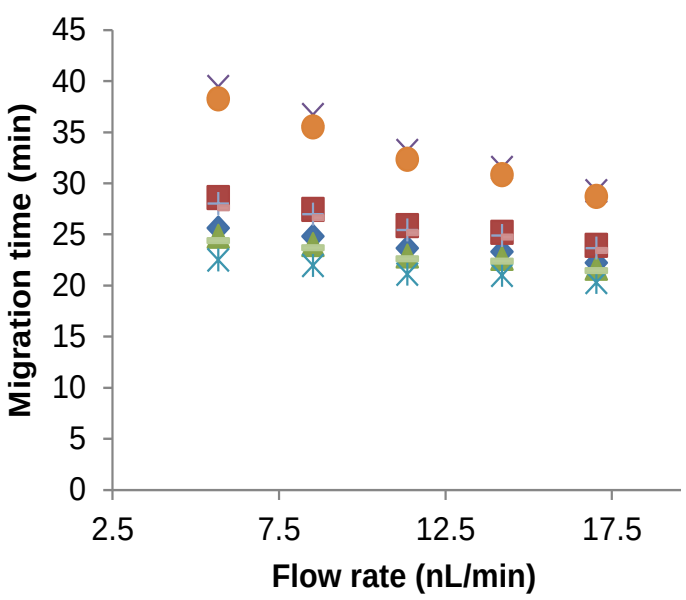
ESI flow rate: 5.7 nL/min; Sample: a mixture of 9 peptides at 2.0 μM each in leading electrolyte; Labeled peaks from 1 to 9 are Kemptide, Substance P, Bradykinin, Angiotensin I, Angiotensinogen (1-14), Neurotensin, Angiotensin II, Leucine Enkephalin, Fibrinopeptide A, respectively.

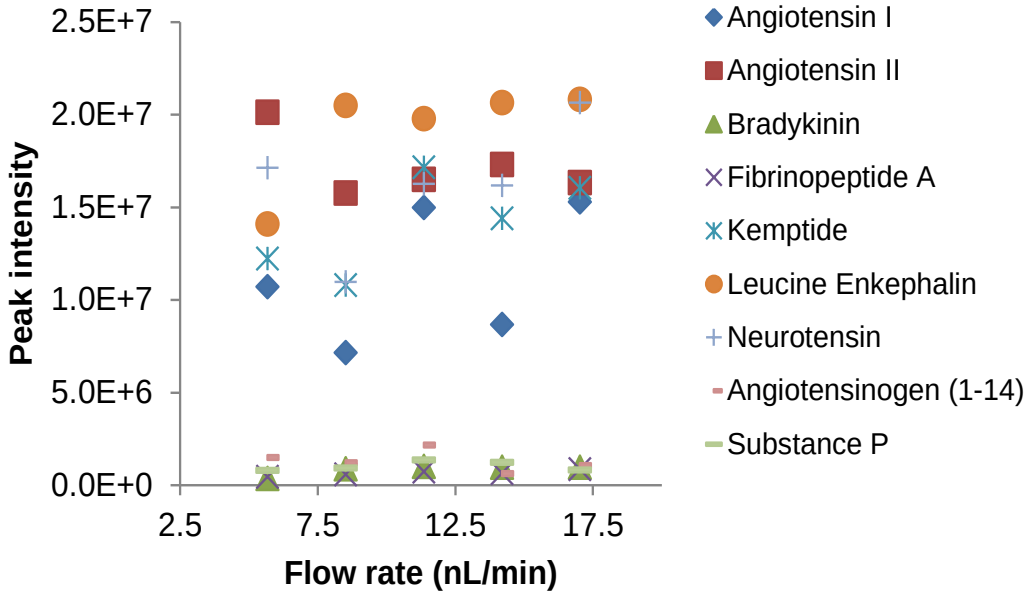
Effect of sample injection volume on peak area



ESI flow rate: 5.7 nL/min; CE separation voltage: 30 kV; ESI voltage: 2.2 kV.

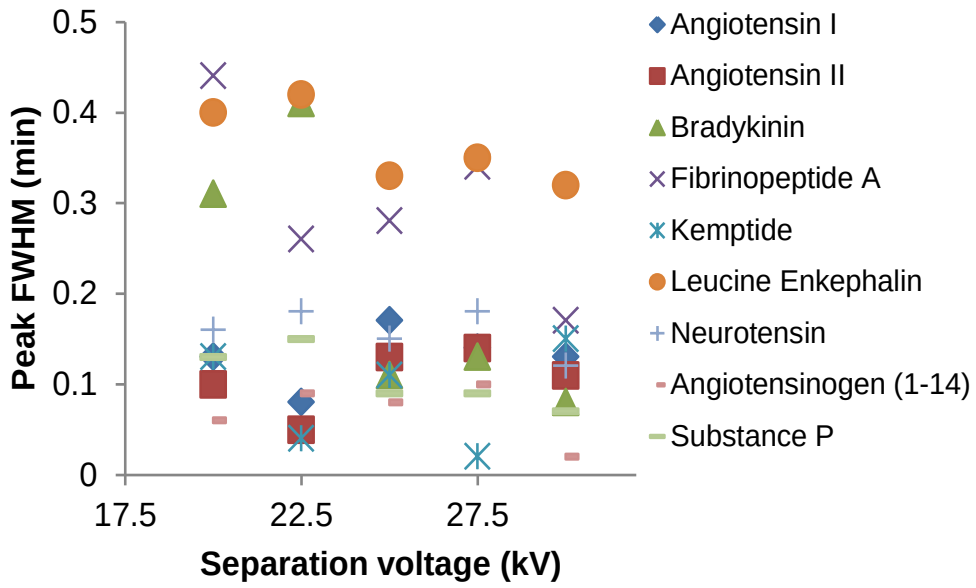
Effect of ESI flow rate on analyte peak migration time and peak intensity

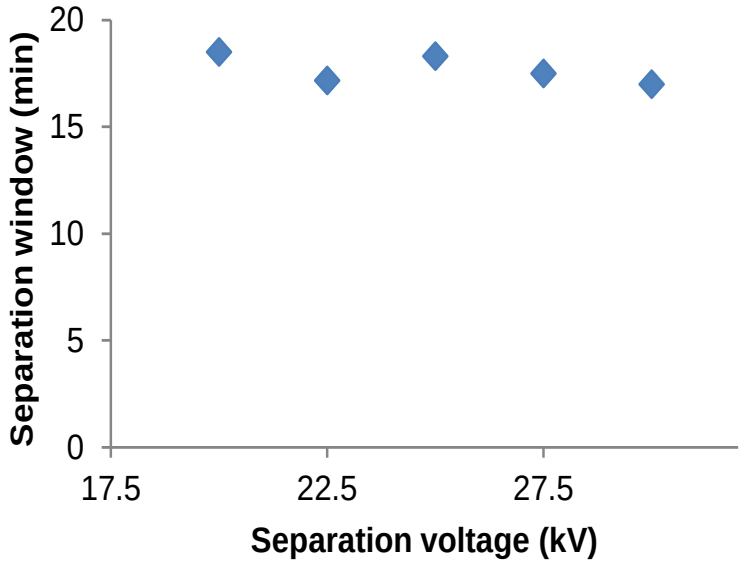




Sample loading volume: 28.4 nL; CE separation voltage: 30 kV; ESI voltage: 2.2 kV.

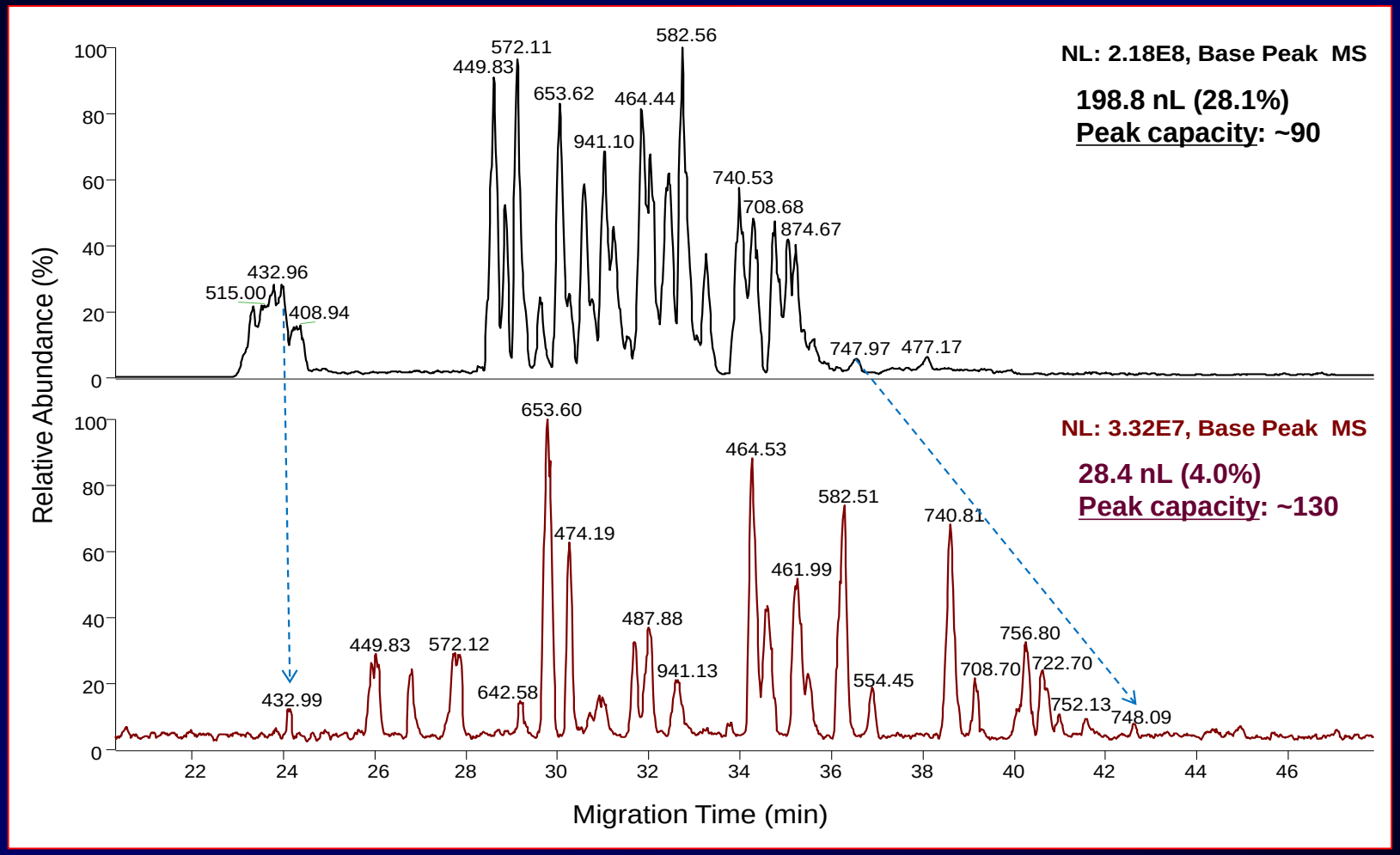
Effect of separation voltage on analyte peak width and total separation window





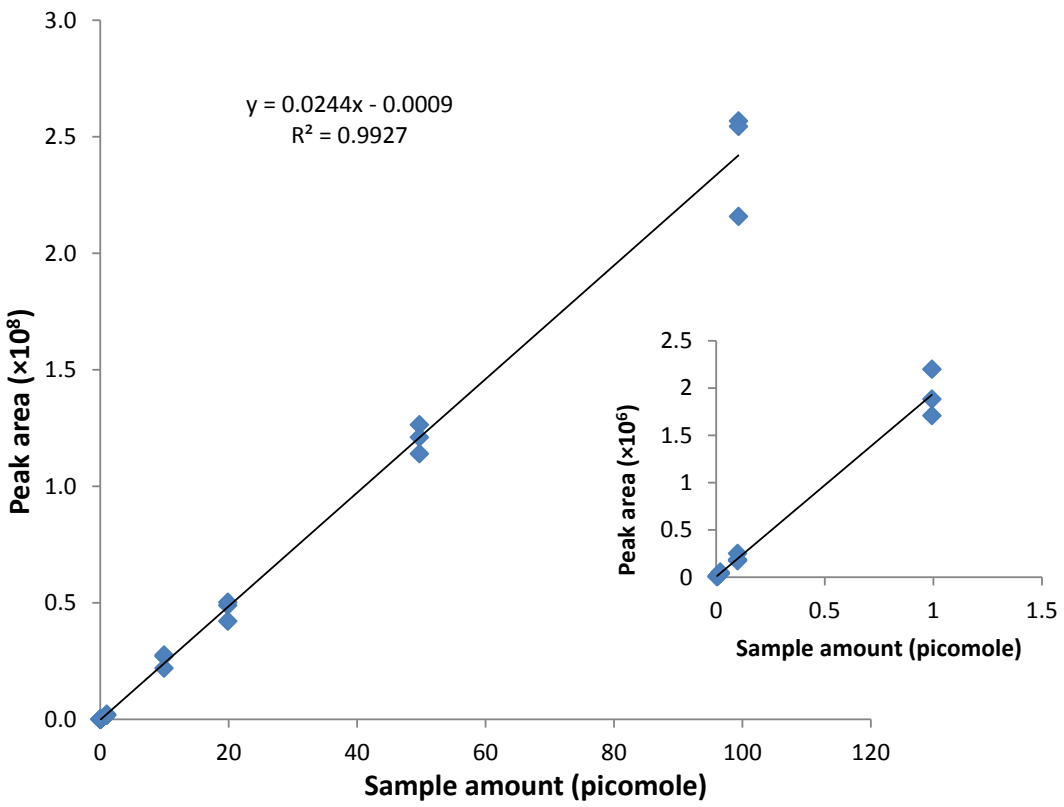
Sample loading volume: 28.4 nL; ESI flow rate: 5.7 nL/min; ESI voltage: 2.2 kV.

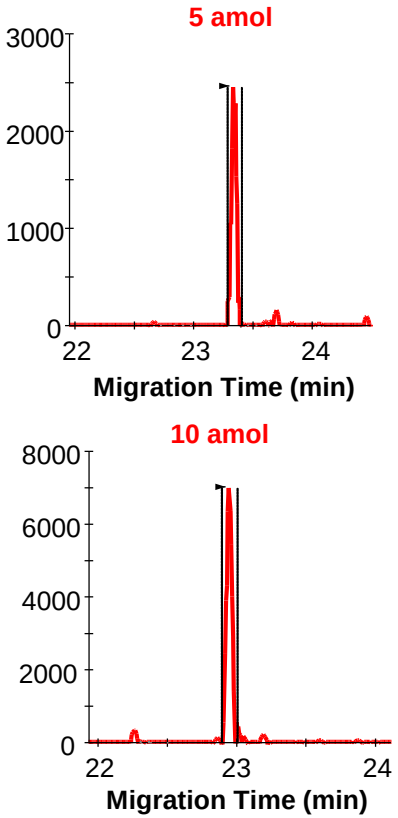
CITP/CZE-MS analyses of 50 nM BSA tryptic digest at different sample injection volumes



ESI flow rate: 5.7 nL/min; ESI voltage: 2.2 kV; CE voltage: 30 kV.

CITP/CZE-SRM MS quantitation of Kemptide





ESI flow rate: 5.7 nL/min, ESI voltage: 2.2 kV, CE voltage: 30 kV, Sample loading volume: 198.8 nL.

Targeted peptides (spiked in 50 nM BSA digest matrix) and monitored transitions			
Compound Name	Sequence	Precursor Ion (m/z)	Product Ion (m/z)
Kemptide	$[LRRASLG+2H]^{2+}$	386.7	567.3 (b_5^+-NH_3), 409.3 (b_3^+-NH_3), 539.4 (a_5^+-NH_3)

Sensitivity, reproducibility and linearity of the CITP/CZE-SRM MS quantification of Kemptide

Concentration (nmol/L)	Amount (amol)	Average migration time (min)	CV of migration time	Average area	CV of area
0.025	5	23.1	0.7%	614×10^3	25.2%
0.05	10	22.5	1.3%	2.14×10^4	5.0%
0.1	20	23.3	0.6%	4.35×10^4	24.6%
0.5	99	22.8	1.8%	2.02×10^5	20.3%
5	994	24.7	0.4%	1.93×10^6	12.9%
50	9.94×10^3	23.7	0.5%	2.55×10^7	12.3%
100	1.99×10^4	23.0	0.2%	4.71×10^7	9.2%
250	4.97×10^4	22.7	0.6%	1.21×10^8	5.2%
500	9.94×10^4	23.3	0.7%	2.42×10^8	9.5%

- Robust new sheathless CE-MS interface: the stability of system remained after more than a hundred sample analyses without the noticeable loss of the metal coating on the emitter surface.

Conclusions:

- The new sheathless CITP/CZE-MS interface design allows robust and reproducible CE-MS analysis.
- The new interface allows stable and dilution free nanoESI operation at the flow rate as low as 5.7 nL/min, improving both CITP/CZE separation quality and MS detection sensitivity.
- The durability of the new sheathless CITP/CZE-MS interface was verified by over a hundred sample analyses without the noticeable loss of the metal coating on the emitter surface and the clogging of the emitter.
- High sensitivity CITP/CZE-NanoESI SRM MS quantification of targeted peptides in BSA digest matrix was demonstrated with LOQ as low as 5 attomoles.

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