Effective Coupling of CITP/CZE with NanoESI-MS using Advanced Interface Technologies for High Sensitivity Sample Quantitation

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Overview

- Novel sheathless interface featuring large sample loading capacity and nanoESI operation was developed.
- Online integration of capillary isotachophoresis /capillary zone electrophoresis (CITP/CZE) with nanoESI MS was employed for selective enrichment of low abundance analyte in a sample mixture.
- Stable nanoESI operation was demonstrated and the influence of flow rate on the instrument performance was systematically investigated.
- Ultrasensitive quantitation can be achieved by sheathless CITP/CZE-MS

Introduction

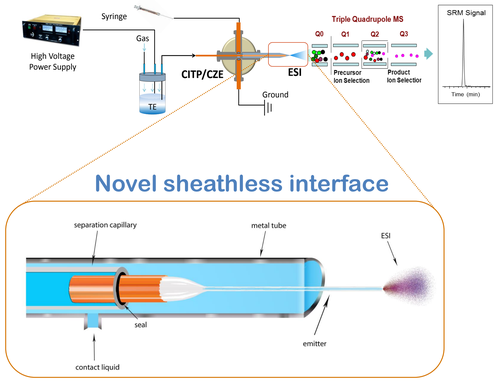
Capillary electrophoresis (CE) coupled with mass spectrometry (MS) is well recognized as an effective technique for chemical and biological sample analysis due to its fast separation speed and high resolving power.

A major limitation affecting its achievable sensitivity is a robust interface that effectively couples CE with electrospray ionization (ESI) MS allowing high sample loading capacity, minimum sample dilution and high efficiency nanoESI operation.

In this study, we presented the development of a new sheathless CITP/CZE-MS interface that effectively resolves the mismatch between the need to use large i.d. separation capillary for large sample loading and small i.d. emitter capillary for nanoESI operation in all the existing interfaces.

Detailed characterization of the new interface was performed to show its achievable sample loading capacity, separation peak capacity, reproducibility and detection sensitivity. The use of the new sheathless CITP/CZE-MS to quantify targeted peptides in complex biological matrix is also systematically evaluated in the study.

Online CITP/CZE-nanoESI-SRM MS[1]



- Large i.d. separation capillary (360 µm o.d./ 100 µm i.d.) is joined with a small i.d. ESI emitter capillary (90 µm o.d./ 20 µm i.d.).
- A section of the emitter capillary (~3 cm long) is chemically etched to porous[2].
- The joint is enclosed in a short metal tube filled with conductive liquid for electric contact, and a high voltage can be applied to the metal tube for nanoESI operation.

Advantages

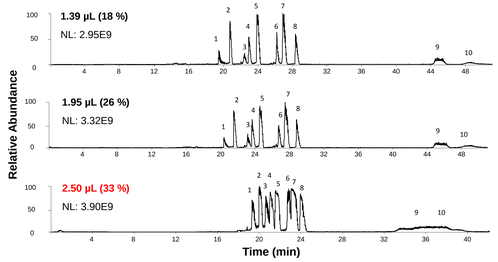
- Large sample loading capacity
- nanoESI operation
- Free of sample dilution
- Flexible setup

Target peptides and SRM monitored transitions

Compound Name	Sequence	Precursor Ion (*m/z*)	Product Ion (*m/z*)
Leu-enkephalin	[YGGFL+H]$^+$	556.3	397.2 (a_4^+), 425.2 (b_4^+), 278.1 (b_3^+)
Angiotensin II	[DRVYIHPF+2H]$^{2+}$	523.8	263.1 (y_2^+), 784.5 (b_6^+), 647.4 (b_5^+)
Kemptide	[LRRASLG+2H]$^{2+}$	386.7	567.3 (b_5^+-NH_3), 409.3 (b_3^+-NH_3), 539.4 (a_5^+-NH_3)
BSA peptide II	[HLVDEPQNLIK+2H]$^{2+}$	653.4	712.4(y_6^+), 251.2(b_2^+), 1056.7(y_9^+)

Results

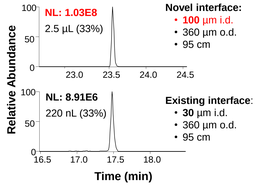
Large sample loading capability



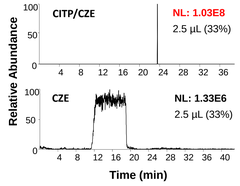
Labeled peaks are 1) melittin, 2) kemptide, 3) substance p, 4) bradykinin, 5) angiotensin I, 6) renin, 7) neurotensin, 8) angiotensin II, 9) leu-enkephalin, and 10) fibrinopeptide A.

Improved sensitivity

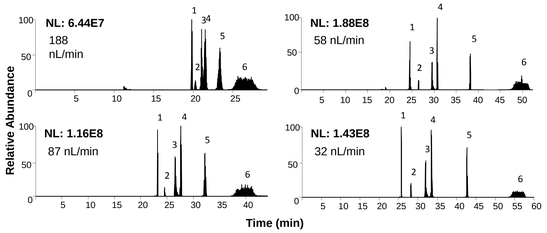
New interface improves loading capacity and sensitivity



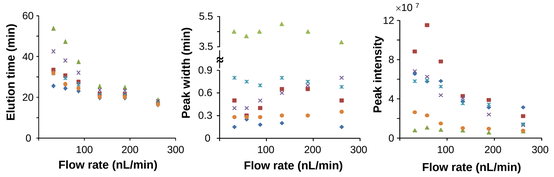
CITP/CZE improves sensitivity and increases sample loading



Flow rate and peak capacity evaluation



Labeled peaks are 1) kemptide, 2) BSA peptide III, 3) BSA peptide II, 4) angiotensin II, 5) BSA peptide I, and 6) leu-enkephalin.

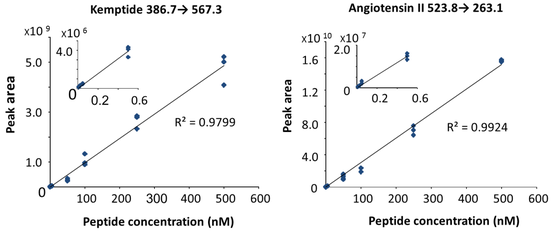


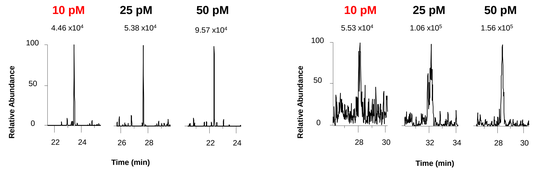
kemptide (◆), angiotensin II (■), leu-enkephalin (♣), BSA peptide I (✖), BSA peptide II (✱), and BSA peptide III (●)

Peak capacity of sheathless CITP/CZE separation at different flow rates

Flow rate (nL/min)	Peak width at half height (min)					Average	Separation window (min)	peak capacity
	kemptide	BSA peptide III	Angiotensin II	BSA peptide I	BSA peptide II			
32.0	0.055	0.090	0.137	0.130	0.100	0.102	28	273
58.0	0.085	0.099	0.074	0.131	0.130	0.104	23	222
87.0	0.068	0.105	0.154	0.180	0.138	0.129	14	109
133	0.055	0.117	0.186	0.340	0.095	0.159	6	37.8
188	0.070	0.130	0.206	0.210	0.092	0.142	5	35.3
261	0.031	0.095	0.247	0.330	0.175	0.176	3	17.1

Ultrasensitive targeted quantitation





Concentration (nM)		0.010	0.025	0.05	0.5	5	50	100	250	500
CV (%)	Kemptide	12	8	6	11	22	15	18	9	10
	Angiotensin II	1	8	22	8	6	21	10	7	1

Conclusions

- With the implementation of the new sheathless interface, the sample loading volume of CITP/CZE can be increased to 2.5 µL, making it comparable to that of a typical nanoLC separation.
- Stable and dilution free nanoESI was demonstrated on the sheathless CITP/CZE-ESI MS showing both a improved separation quality and detection sensitivity.
- High sensitivity CITP/CZE-NanoESI SRM MS quantitation of targeted peptides in a complex sample was demonstrated with LOQ as low as 10 pM at total sample loading of 25 attomoles.

Acknowledgements

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References

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