Fast screening and characterization of therapeutic peptide by online capillary electrophoresis - mass spectrometry (CE-MS)

Fuxing Xu[1, *], Zhe Li[1], Ling Ling [1],Liang Wang[2], Kate Yu[2]

[1.]Ningbo university, Ningbo, 200032, China; [2.]908Devices Inc. Boston, MA, 02210, USA

Overview

Peptides mainly refer to molecules containing two to fifty amino acids linked by peptide bonds and molecularly poised between small molecules and proteins. Therapeutic peptides are a unique class of pharmaceutical agents composed of a series of well-ordered amino acids, usually with molecular weights of 500-5000 Da.

Therapeutic peptides have several important advantages over proteins or antibodies: they are small, easy to synthesize and can penetrate the cell membranes. They also have high activity, specificity and affinity; minimal drug-drug interaction; and biological and chemical diversity.

However, disadvantages related to peptide drugs are still commonly present. In comparison to chemical drugs, some shortages of therapeutic peptides include unstable physicochemical properties, rapid oxidation and hydrolyzation, upregulated agglomeration, shorter half-life, higher clearance rates, and downregulated permeability toward cell membranes. Therefore, peptide drug development and production processes are complicated and confront various unknown circumstances.

As a result, a comprehensive characterization analysis must be performed to ensure safe and effective therapeutic peptide productions. Here we present a quick characterization workflow of therapeutic peptides based on microchip CE-MS.

Methods

- A novel strategy based on CE-MS workflow was utilized for this work combining fast CE separation with high-resolution mass spec identification.

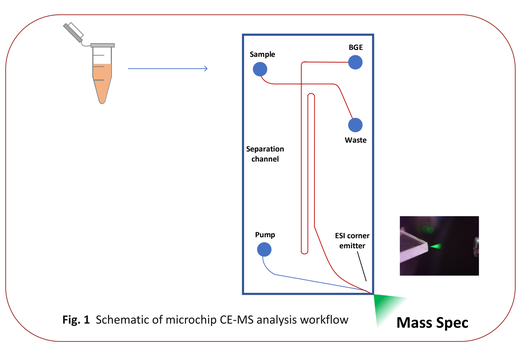

Fig. 1 Schematic of microchip CE-MS analysis workflow

Methods

- Peptide samples including therapeutic peptides were firstly diluted in water and followed by dilution with organic solvent containing methanal or acetonitrile with ammonium acetate.
- CE separation was implemented through a 22 cm length CE separation channel (ZipChip^{TM} HR chip) and 10 cm length separation channel (ZipChip^{TM} HS chip) in a conductive liquid BGE solution composed of the mixture of acetonitrile and water (ZipChip^{TM} Peptide Assay Kit)
- Two different type mass spectrometer were utilized to coupled with microchip CE interface, which are a Q-TOF MS from Bruker (timsToF) and Orbitrap MS from Thermo Fisher (QExactive).
- Only positively charged components in the sample can be analyzed based unique microchip CE principle.

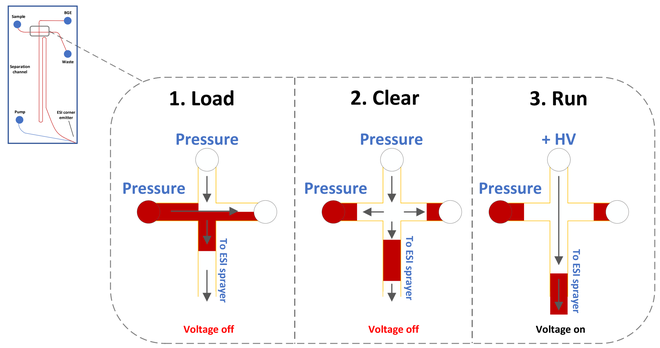

Fig. 2 The pressure-driven injection scheme of microchip CE

◆ Unique sample injection and separation from microchip CE

- **Injection**: a novel sample injection method that utilizes pressure-driven flow to precisely deliver aliquots of sample into the separation channel with no injection bias. To introduce sample into the separation channel as **Load** step, gas pressure is applied to chip wells, nanoliter sample volumes were delivered into chip channel based on a precisely controlled loading duration. The following up is a **Clear** step utilizing the flow of clean BGE to form a cleanly defined plug of sample in the separation channel. At this point voltage is applied to perform the electrophoretic separation **Run**.

- **Separation**: Immediately following the sample injection sequence, electrical voltage more than 10 KV is applied across both ends of the separation channel. These voltages dictate the electrical field strength for the CE separation and the ESI voltage. Analytes migrate down the separation channel in the electric field toward the ESI orifice where they are then ionized and introduced into the mass spectrometer. The ESI voltage was applied to the chip corner with a ~100um distance, indicating a very small dead volume compared to traditional capillary-based CE.

Results

◆ Fast intact mass identification

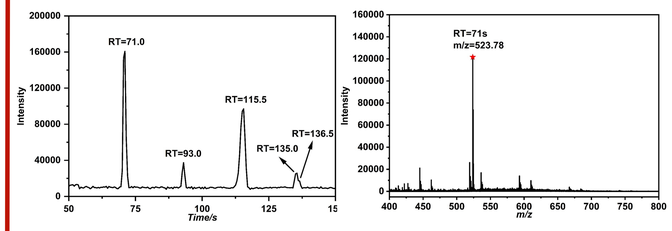

Fig. 3 Microchip CE separation spectrum and MS analysis of a peptide mixture sample

- In less than 150 seconds, 5 peptides with different length were well separated in CE separation, the mass spectrum of octapeptide shows a very clean background and good S/N response.
- The peptide with more positive charged migrated fast and can be obtained within less then 1 minutes.
- Data was acquired by both Q-TOF and Orbitrap mass spec successfully. The data shown here is from Q-TOF MS.

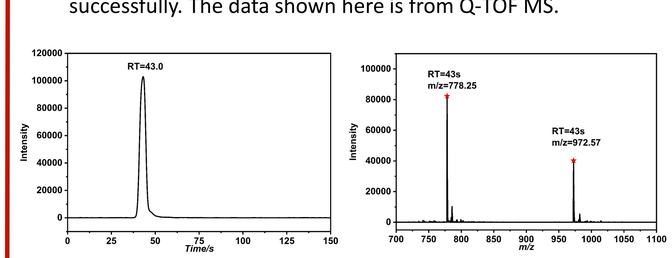

Fig. 4 Microchip CE separation spectrum and MS analysis of a therapeutic peptide sample

- In less than 60 seconds, a therapeutic peptide with molecular weight of 3886Da, including 34 amino acids, was well characterized by microchip CE with a sharp and narrow migration peak.
- The mass spectrum shows a clean background and accurate m/z value. The concentration is 1.0 µg/mL.
- Only positively charged components in the sample can be analyzed based unique microchip CE principle.
- Data was acquired by both Q-TOF and Orbitrap mass spec successfully. The data shown here is from Q-TOF MS.

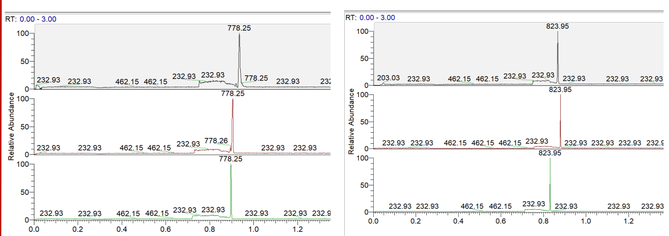

Fig. 5 CE migration spectrum of two therapeutic peptide samples.

- The reproducibility for microchip CE were performed and 3 replicates for two therapeutic peptide samples were analyzed and the migration time matched very well.
- Data was acquired by both Q-TOF and Orbitrap mass spec successfully. The data shown here is from Orbitrap MS.

Results

◆ Quantification analysis

- Therapeutic peptide samples were diluted to different concentration from 1-100ug/mL and 1-250ug/mL, without any further sample preparation work.

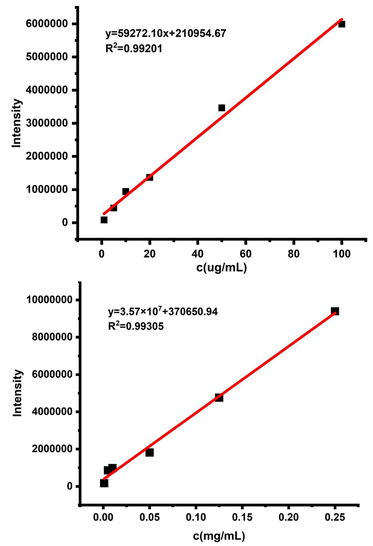

Fig. 6 Quantitative Standard curve for different concentration from two peptide samples.

- A very good linearity response were achieved for both therapeutic peptides.
- The results bring this method good capability as a DMPK method for therapeutic peptides.

Summary

- A novel strategy for characterization of therapeutic peptide was developed based on microchip CE-MS and linkage-specific derivatization.
- And successfully qualitative and quantitative analysis of therapeutic peptide samples including 34 and 21 amino acid polypeptides, demonstrated a high-throughput and straightforward workflow for in-depth characterization of peptide based therapeutic drugs.
- Tandem mass spectrometry for structure elucidation have been explored as well and very promising result were achieved.

References

1. *Sig Transduct Target Ther* **7**, 48 (2022).
2. Metabolites 2022, 12, 532
3. ZipChip TechNote 1.0