An Advanced CE-QTOF Technique For The Rapid Characterization Of Amino Acids In Herbal Medicines

Tao Bo, Zhengxiang Zhang, Xiaorong Ran, Jianzhong Li, Agilent Technologies, No.3, Wang Jing Bei Lu, Chao Yang District, Beijing, 100102, China
Introduction

Amino acids in Chinese traditional medicines (TCMs) are important bio-active components which have attracted much attention. It is crucial to develop high-throughput and high-selective technology for simultaneously screening multiple amino acids in complex matrices. Capillary electrophoresis (CE) is a robust separation technique based on electrophoretic migration which has been widely used for analyzing TCMs.

Quadrupole-time of flight (QTOF) mass spectrometry with high resolution, accurate mass and MS/MS capability plays a crucial role in the analysis of TCMs because structural information including the accurate mass can be determined for both precursor and product ions.

In this study, a high throughput CE-QTOF tandem technique with very good sensitivity and reliable confirmation was developed for characterizing sixteen amino acids in herbal medicines. The method uses systematic validation, involving data processing (Mass Feature Extract, MFE), accurate mass database search, and structural confirmation based on MS/MS fragmentation.

Experimental

Separation conditions
Separation was performed using the Agilent 7100 HPCE using 1 M formic acid as running buffer. The sheath liquid flow rate was 4μL/min with 5mM acetate ammonium in 50% methanol. Applied voltage was set at 30 kV and capillary temperature was set at 25°C. The effective capillary length was 80 cm with 50 μm ID.

MS conditions
Mass system : Agilent 6550 QTOF
Ion source: Jet Spray ESI
Nebulizer gas: N₂
Polarity: positive mode
Nebulizer pressure: 10.0 psi
Drying gas temperature: 280 °C
Drying gas flow rate: 10.0 L/min
Sheath gas: 5L/min at 250°C
Scan mode: full scan and targeted MS/MS
Mass range: 100-1000 m/z
Internal mass calibration: Calibration using two reference ions

Sample preparation
Sixteen amino acid standards and herbal medicine samples (Radix Astragali Injection) for this study were obtained from the Shanghai Institute of Drug Control.
An Advanced CE-QTOF Technique For The Rapid Characterization Of Amino Acids In Herbal Medicines

Results and Discussion

Optimization of CE
An optimized method for the rapid analysis of amino acids in TCM samples by CE-QTOF has been developed. Using an electrolyte with a pH value below the analyte’s isoelectric point (< 2.77) allows the simultaneous analysis of sixteen amino acids as positive molecular ions. The conditions of CE were systematically optimized for running buffer system and sheath liquid components to obtain a highly efficient and fast separation. Especially, leucine and iso-leucine are well separated using the optimized conditions in the Radix Astragali Injection samples as shown in Figure 1.

Identification of amino acids by QTOF
The mass spectrometry parameters were optimized for best sensitivity with Jet Spray ESI, Fragmentor voltage and acquisition rate. The identification and fragmentation mechanism of amino acids is illustrated for proline using MS and targeted MS/MS are shown in Figure 2.

Figure 1: Optimized separation of amino acids by CE-QTOF

Figure 2: The identification and fragmentation mechanism by MS and targeted MS/MS
Results and Discussion

TCM injection analysis and method validation

Figure 3 displays the analysis of amino acids in TCM samples by CE-QTOF with high throughput and high sensitivity. A TCM database (containing 10,463 active compounds) based on accurate mass was used for rapid amino acid screening after the MS data was processed by the MFE algorithm to remove the background ions and generate a compound list (as shown in Figure 4). Migration time and peak area reproducibility were excellent (<5.0% RSD for n = 3) as shown in Figure 5. Detection limits ranged from 0.5-10 µg/mL for all analytes.

![Figure 3: High throughput analysis of amino acids in the TCM sample by CE-QTOF.](image)

![Figure 4: TCM database containing 10,463 active compounds was used for rapid amino acid screening.](image)

![Figure 5: Excellent migration time reproducibility and peak area as exemplified for proline.](image)

Conclusions

The study demonstrates the use of a high sensitivity and selectivity CE-QTOF method for high throughput amino acid screening in TCMs.

In addition, the established CE-QTOF method can be applied to profiling amino acids in other complex matrices such as food and serum.