

## 5 - 66 Establishment of a Analytical Method for Bioactive Polypeptides Structure on LC-MS/MS

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Bioactive polypeptides are intermediate products of different sizes formed by enzymatic hydrolysis of proteins, which have more stable processing properties and more easily absorbed physiological properties than proteins<sup>[1]</sup>.

To study the structure of polypeptides, high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) was applied, and auto MS/MS method was established<sup>[2]</sup>. Samples were resuspended in 200  $\mu\text{L}$  2 % ACN, 0.1 % formic acid, with a total of 5  $\mu\text{L}$  injected. The ESI-LC-MS/MS was used to analyse the peptide samples. The LC system was set up with an C18 analytical column and controlled at 35 °C. Solvent A was 0.1 % v/v formic acid and solvent B was 0.1 % v/v formic acid and 5 % ACN. The gradient settings for the LC runs, at a flow rate 200  $\mu\text{L}$  /min, were as follows: solvent B 2 % to 35 % in 2 min, 35% to 90 % in 10 min, and equilibration at 2% solvent B for 2 min. A positive mode, was employed, and the mass spectrometry data was acquired with Auto MS/MS mode. Full scan MS spectra features ions at 150~2 200M/Z. The results show that the precise molecular and fragment ions were detected(Fig.1).

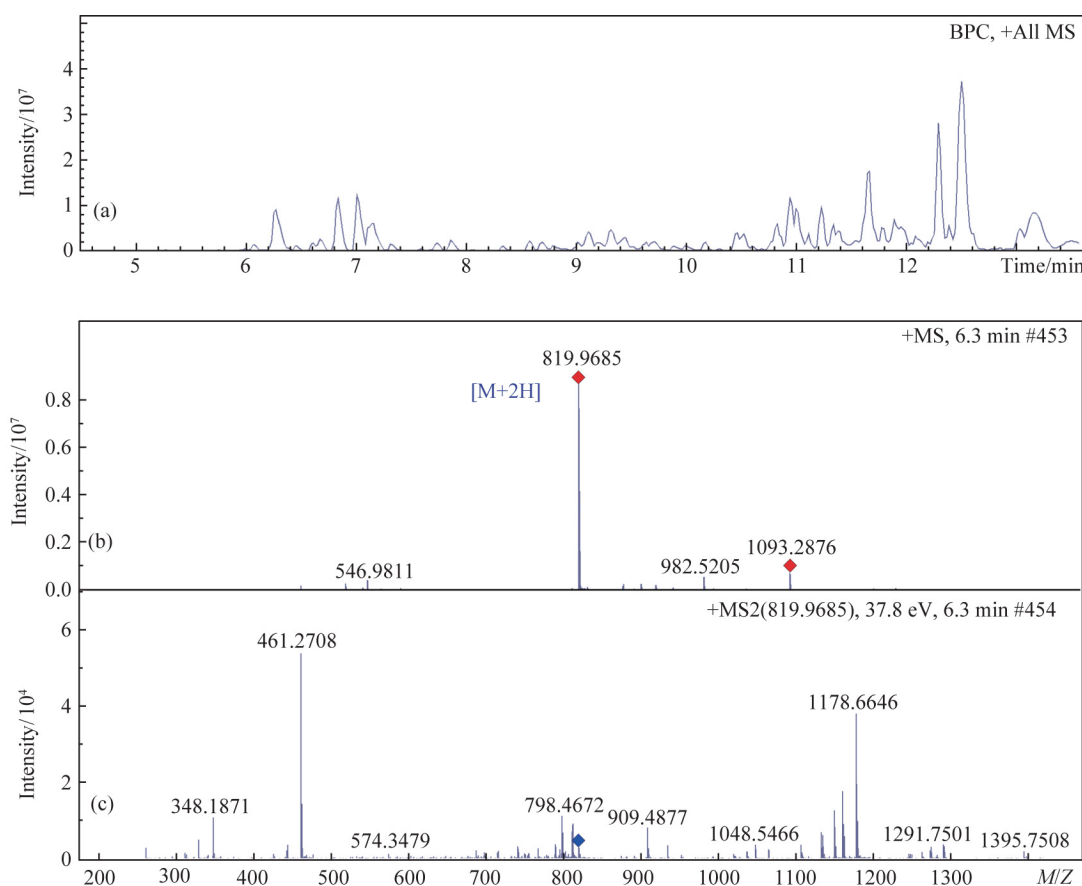


Fig. 1 (color online) (a) LC-MS Chromatogram, (b) ESI-MS spectra of the bioactive compound with precise molecular, (c) Fragment ions of bioactive compound(819M/Z).

### References

- [1] R. Y. Zang, Q. L. Yu, Z. L. Guo, et al., *Foods*, 2, 2(2023)407.
- [2] Ortiz-Lopez, Carretero-Molina, Sanchez-Hidalgo, et al., *Angewa. Chemie. Int. Ed.*, 59, 31(2020)12654.