Profiling and Characterization of Polyphenol Polymers from Cinnamon Using an Ion Trap Mass Spectrometer

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**Goal:** To develop a rapid and rugged LC/MS\textsuperscript{n} method for high throughput characterization of the active components in cinnamon extract.

**Introduction**

Type 2 diabetes is the most common metabolic disease worldwide. Although the prevention and control of it remains unclear, there is strong evidence that dietary factors play a role in the onset of this disease. Previous studies have indicated that cinnamon may mimic insulin effects and thus may improve glucose uptake.\textsuperscript{1,2} Considering the complex composition of cinnamon, it is important to be able to isolate and characterize the possible active components in it. A range of reports have been devoted to the studies of this topic. However, most work has been mainly focused on the studies of monomer and dimers. The study of trimer or even larger compounds using on-line LC/MS\textsuperscript{n} approach has not been reported.

The fast cycle time and excellent MS\textsuperscript{n} sensitivity of the LCQ Fleet ion trap mass spectrometer provide enhanced analytical throughput for complex natural product analysis. In this report, profiling and structural elucidation of polyphenol polymers, the possible active compounds in cinnamon, are carried out using an LC/MS\textsuperscript{n} methodology using the LCQ Fleet ion trap mass spectrometer. The application of pulsed-Q dissociation (PQD\textsuperscript{™}) and high resolution isolation (HRI) facilitates the analysis of this complex sample.

**Methods**

An aliquot (0.5 g) of cinnamon was ground and extracted in 8 mL 0.1 N acetic acid using a sonic bath for 3 minutes. The supernatant liquid was cooled to room temperature and diluted in mobile phase. The final solution was filtered prior to HPLC separation. LC separation was performed using a C18 column and detected at 280 nm wavelength. Sample fractions CB4 with insulin enhancing-activity were collected, and the powders were dissolved in water:MeOH:ACN = 25:50:25 solvent for LC-MS\textsuperscript{n} analysis using the LCQ Fleet ion trap mass spectrometer.

**LC instrument:** Surveyor Plus LC system

**Column:** Hypersil GOLD (50 × 2.1 mm, 3 µm particle size, Thermo Fisher Scientific)

**Mobil phase:**
- A: Water with 0.1% formic acid
- B: Acetonitrile with 0.1% formic acid

**Flow rate:** 400 µL/min

**Mass Spectrometer:** The LCQ Fleet ion trap mass spectrometer was operated in positive electrospray mode. The electrospray voltage was 5 kV. The capillary temperature was 200 °C and the sheath gas flow was 30 units. An isolation width of 2 Da was used with a 30 msec activation time for MS\textsuperscript{n} experiments. All scan events were acquired with a 200 ms maximum ionization time.
Results

It has been reported that a group of polyphenolic polymers found in cinnamon may function as antioxidants to potentiate insulin action, and therefore, may be beneficial in the control of glucose intolerance and diabetes. Two major types of polymeric procyanidins have been observed including A-type and B-type linkages. Among them, A-type has two interflavan linkages (IFL) while B-type has a single IFL, which results in a 2 amu unit difference. Studies have been performed on both types of moneric, dimeric and trimeric procyanidins. However, detailed structural elucidation results for higher orders of polymerization are still rare, particularly using an on-line LC/MS\(^n\) approach.\(^1\)\(^3\) In this work, a range of procyanidins from cinnamon extract fractions have been identified and characterized within a rapid ten-scan-event LC-MS\(^n\) analysis (Figure 2a). The fast positive/negative switching the capability of the LCQ Fleet mass spectrometer ensured the comprehensive information collection from the samples. Figure 2b and Figure 2c demonstrated the MS\(^n\) (up to MS\(^5\)) data of one trimer procyanidin in both positive (Figure 2b) and negative (Figure 2c) polarities from fraction CB4. The ability to perform MS\(^n\) analysis on precursor ions (isolated using HRI) using the ion trap enables the structural elucidation of these compounds with less interference from the matrices. The presence of \(m/z\) 289 in negative MS/MS spectrum is considered to be specific for a C-O-C IFL in the polymers.\(^4\)\(^5\)

Mass Frontier software contains a number of tools for structural identification. Its fragment prediction module is very useful for accurate structural characterization from MS\(n\) data. As an example, the positive MS/MS spectrum of type A procyanidin trimer was processed with Mass Frontier, and all the major fragments were assigned with a predicted sub-structure (Figure 3). This feature can be further coupled with the database searching of Mass Frontier for compound identification.

Mass Frontier also predicted that the ion at \(m/z\) 287 could be generated from singly linked B-type procyanidin oligomers, which is consistent with the observations in this work. The fragmentation of the ion at \(m/z\) 867 resembles that of \(m/z\) 865 with \(m/z\) 287 as a common fragment ion (data not shown). Therefore, the ion at \(m/z\) 287 may not be a good indicator ion for the presence of A-type procyanidin oligomers, as noted in a previous report.\(^1\)

![Figure 2: LC-MS\(n\) Analysis of doubly linked A-type Procyanidin from Cinnamon.](image)

(a) Ten-scan-event MS\(^n\) method setup: MS\(^n\) was performed on the most abundant ions from the previous level of analysis; (b) MS to up to MS\(^5\) data for the target compound with \(m/z\) 865 in positive polarity; (c) MS to up to MS\(^5\) data for the target compound with \(m/z\) 863 in negative polarity. (The data was collected from fraction CB4)

![Figure 3: Fragmentation prediction for the identification of A-type procyanidin trimer using Mass Frontier](image)
Dimers and tetramers have been found, in addition to the trimers, in this fraction with up to MS^3 data for identification purpose in both polarities. With the traditional CID method, the m/z 289 from MS/MS spectrum of m/z 1151 (the tetramer) in negative polarity is not observable in an ion trap mass spectrometer due to the low mass cutoff (Figure 4a). In order to observe this indicator ion, an MS^3 analysis is usually required (see insert in Figure 4a), which may be impacted by both cycle time and signal strength. With the new dissociation method, Pulsed Q Dissociation (PQD), it is possible to access directly the low mass product ions in the ion trap without the need for the further step of MS^n analysis. As demonstrated in Figure 4b, the ion at m/z 289 was observed directly from the MS/MS spectrum of the ion at m/z 1151.

**Conclusions**

A rapid and rugged LC-MS^n method has been developed to characterize the various procyanidin oligomers in the Chinese cinnamon extract. Both A-type and B-type polymers were found. The fast cycle time and excellent MS^n capability of the LCQ Fleet enabled the confident identification of the components of interest. Comprehensive information was obtained in both positive and negative polarities. Use of PQD provided diagnostic low mass product ions without the need for higher order MS^n experiments.

**References**


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