Application of LC-MS with ESI to Glycosylated Natural Products

Application

LC-MS

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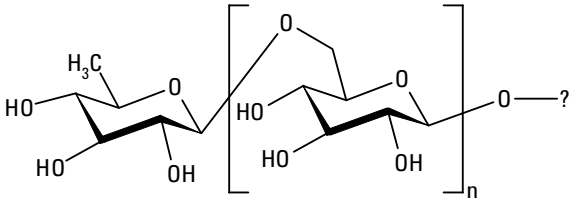
Abstract

The sequencing of oligosaccharides in glycosylated natural products was successfully investigated using liquid chromatography-mass spectrometry with electrospray ion source in negative ion mode.

Background

The sequencing of oligosaccharides is a major goal in the field of glycosylated natural products.



Prior attempts to do so, using liquid chromatography-mass spectrometry (LC-MS) with electrospray ion (ESI) source on samples derived from such products have encountered serious complications. For example, the application of ESI in positive ion mode often leads to extensive formation of [M+Na]+ ions for heavily glycosylated compounds. These ions do not lend themselves to elegant fragmentation for many classes of glycosylated compounds.

The technique described below yields intense negative ions of the $[M-H]^-$ variety, and they possess excellent fragmentation properties, allowing the sequence of the oligosaccharide moiety on the compound to be readily investigated.

Method

- Instrument: Agilent 1100 LC/MS in ESI negative ion mode

  Drying gas: 10 L/min and 350 °C

  Nebulizer: 50 psi

  V_{cap}: 3000 V

  Fragmentor: 60, 100, or 400 V according to experimental needs

- LC Conditions:

  10 mM ammonium acetate vs. acetonitrile mobile phase gradient

  - 10 to 90% acetonitrile in 15 minutes
  - Hold 10 minutes

  Flow rate: 0.6 mL/min. No split.

- Post-column addition:

  50% triethylamine (TEA) in isopropanol (IPA) added at 0.2 mL/min.

- Column:

  ZORBAX 300SB-C18, 2.1 mm id × 15 cm long, 5 µm particles.

A sample application: Characterization of a Hostas extract

Extracts of the Hostas plant containing glycosylated flavanoid compounds, were separately injected into the LC and processed under various experimental conditions. Figure 1 compares the negative ion chromatograms obtained at two fragmentor settings, 60 and 400 V.

The six numbered peaks in Figure 1 were selected for detailed mass spectral study. Increasing fragmentor voltage produces greater molecular fragmentation, changing both the negative ion chromatogram and the mass spectrum of each peak, allowing additional molecular information to be extracted.

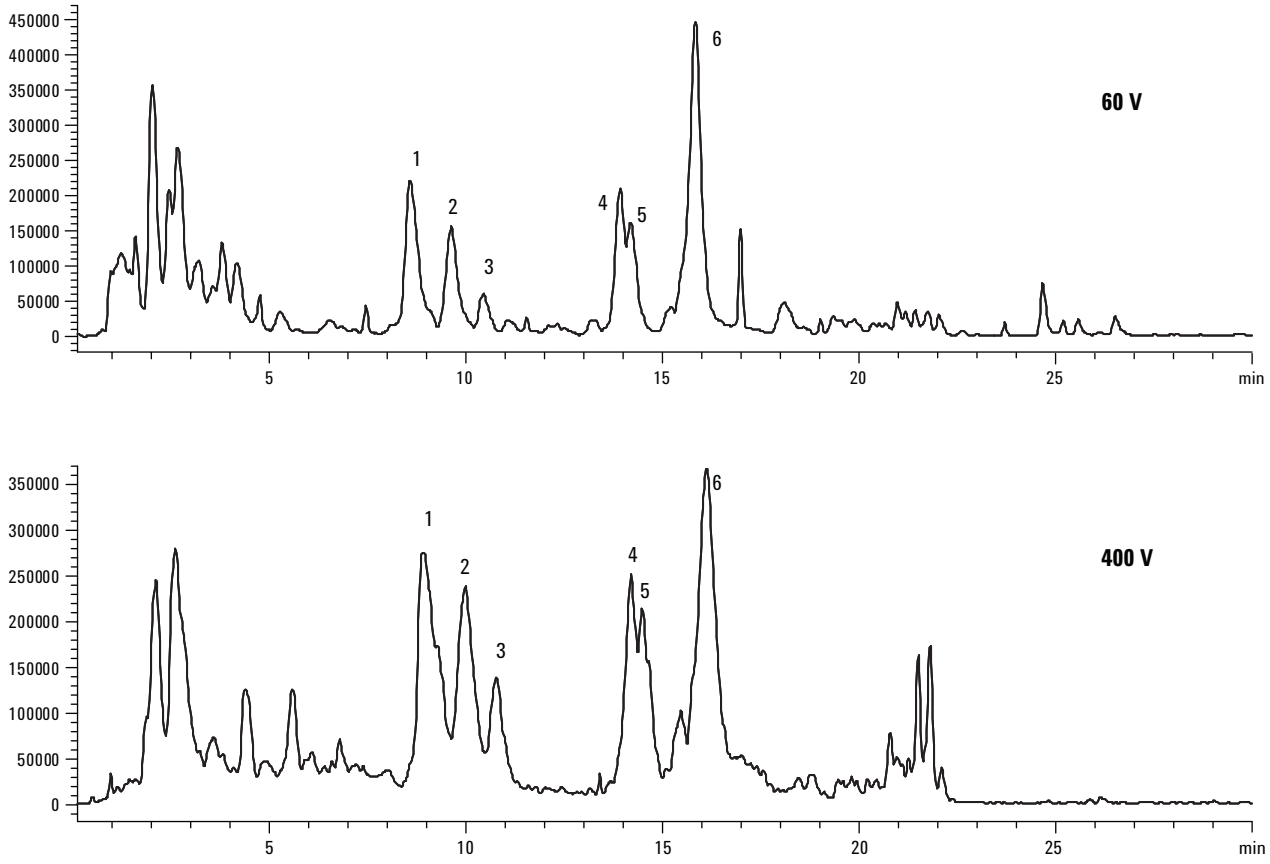


Figure 1. Stacked negative ion chromatograms obtained with the fragmentor set to 60 and 400 V.

Figure 2 shows the mass spectrum of peak 1 at 60 and 400 V.

Note that the peak at 1259.5 *m/z* represents the loss of one glycosyl unit (146 daltons), from the peak at 1405.5 *m/z*. At 60 V the peak at 702.7 *m/z* represents the doubly charged ion of the same parent molecule that produced the singly charged ion at 1405.5 *m/z*.

The mass spectrum obtained at 400 V is very different. Note the increased number of fragment peaks and the mass differences between the major peaks, corresponding to the loss of different numbers of glycosyl units. 162 and 146 daltons represent glycosyl units with and without the ether oxygen respectively.

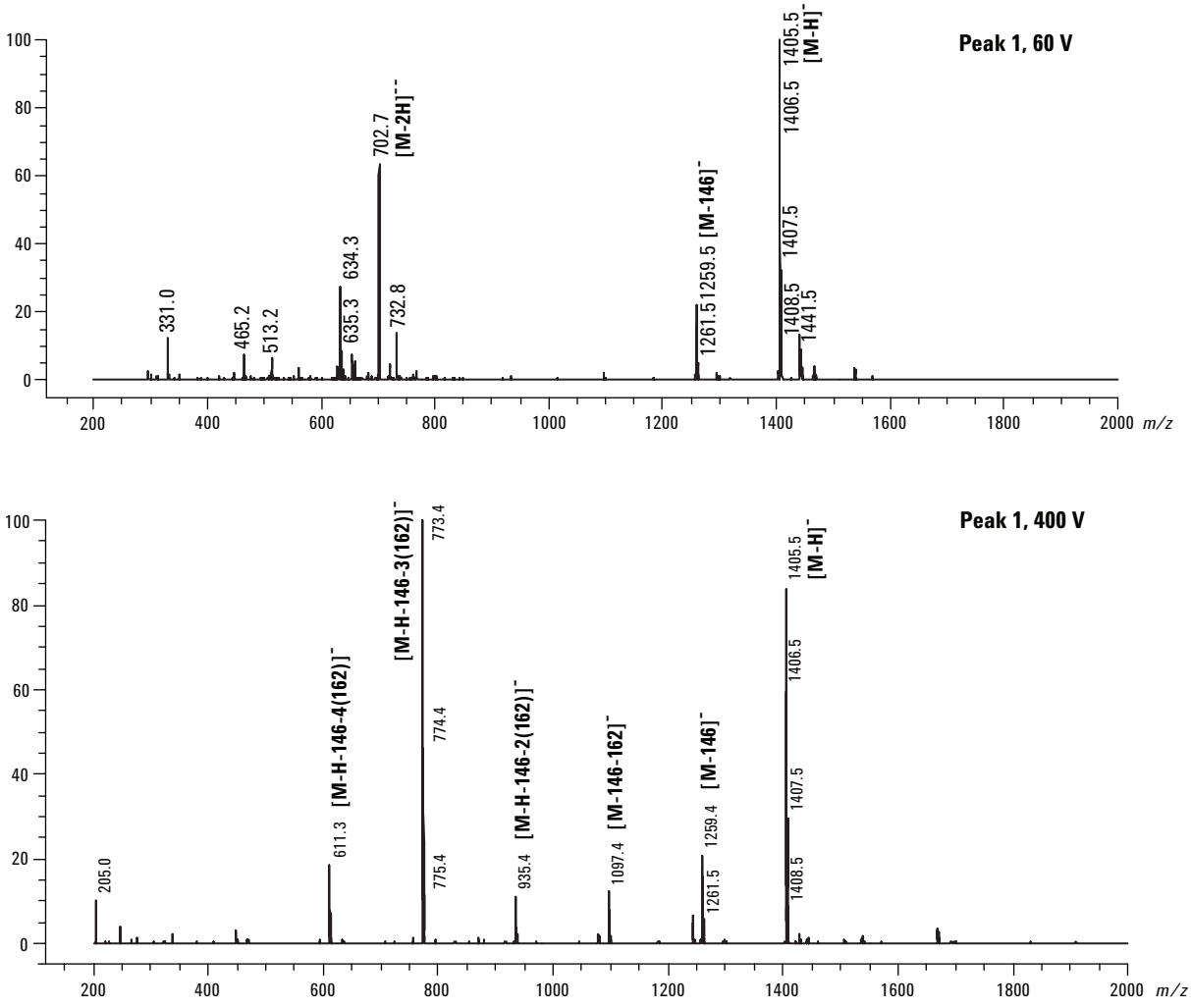


Figure 2. Mass spectrum of peak 1 compared; fragmentor at 60 and 400 V.

Figure 3 shows the mass spectrum for peak 2, at 60 V.

Here we see successive losses of glycosyl units, between the peaks at 1375.5, 1229.5, and 1081.4 *m/z*.

Figure 4 shows the mass spectrum of peak 6, at 60 and 400 V.

Here we note that 60 V is an insufficient setting to provide sequence information and that 400 V is. Note the successive losses of glycosyl units, with and without ether oxygen, between the peaks at 1065.4, 903.5, 757.3, and 595.3.

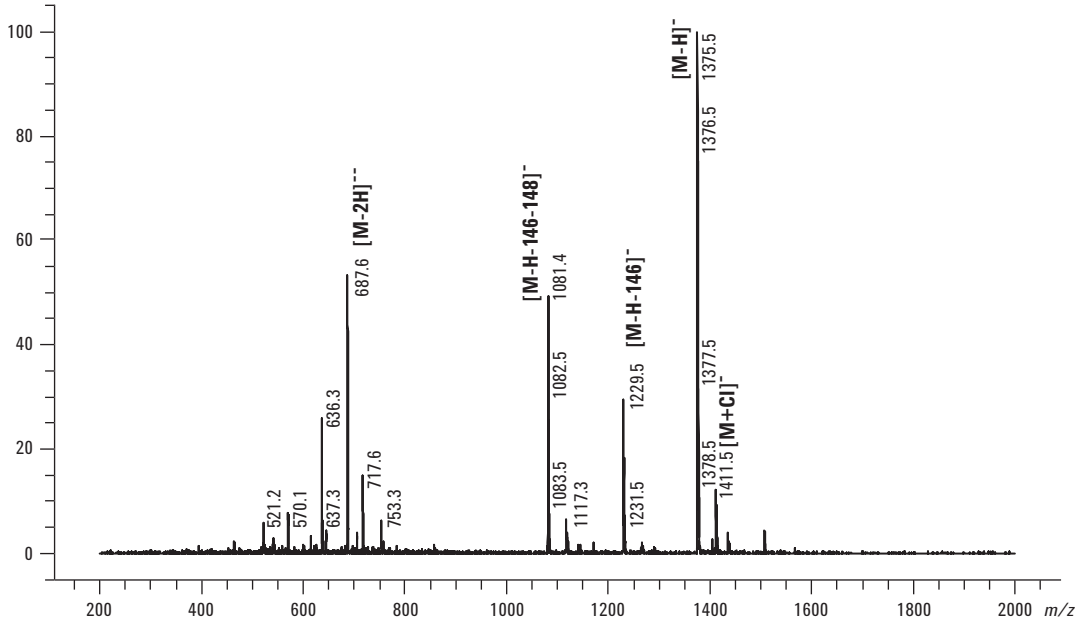


Figure 3. Mass spectrum of Peak 2; fragmentor at 60 V.

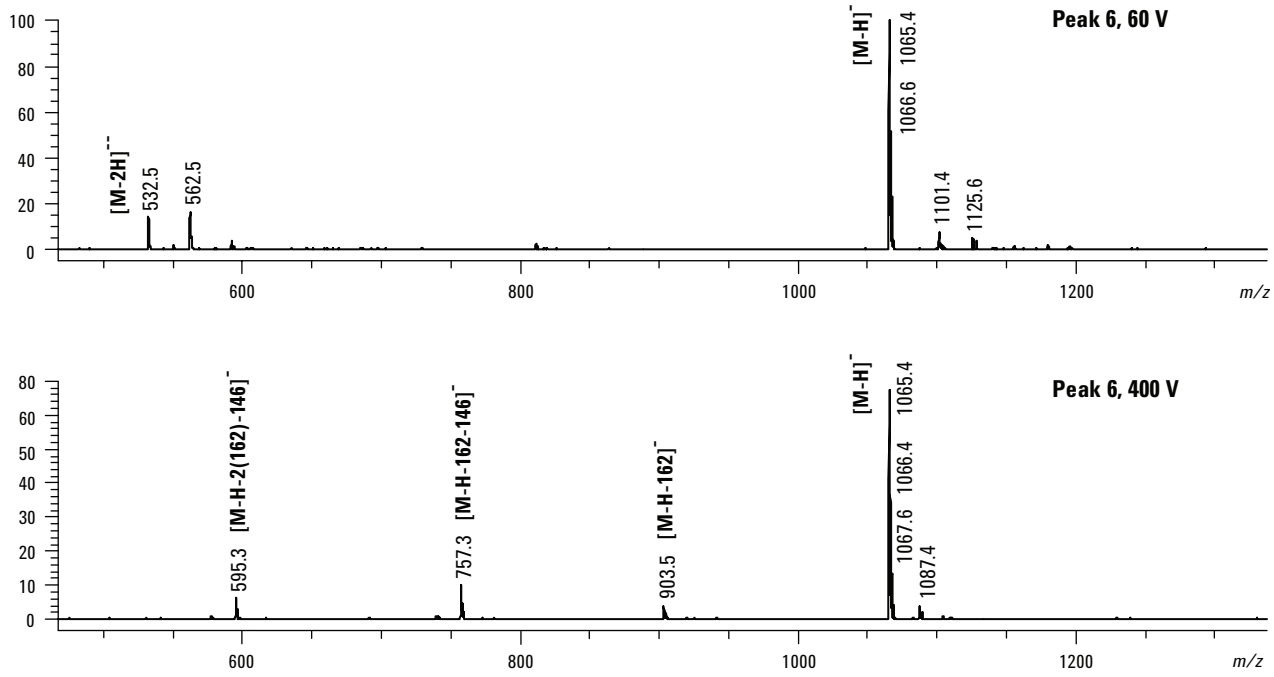


Figure 4. Mass spectrum of peak 6 compared; fragmentor at 60 and 400 V.

The major ions of each of the selected peaks, with fragmentor at 60 V, were extracted and stacked to produce Figure 5, demonstrating our ability to extract suitable ions for detailed mass spectral study.

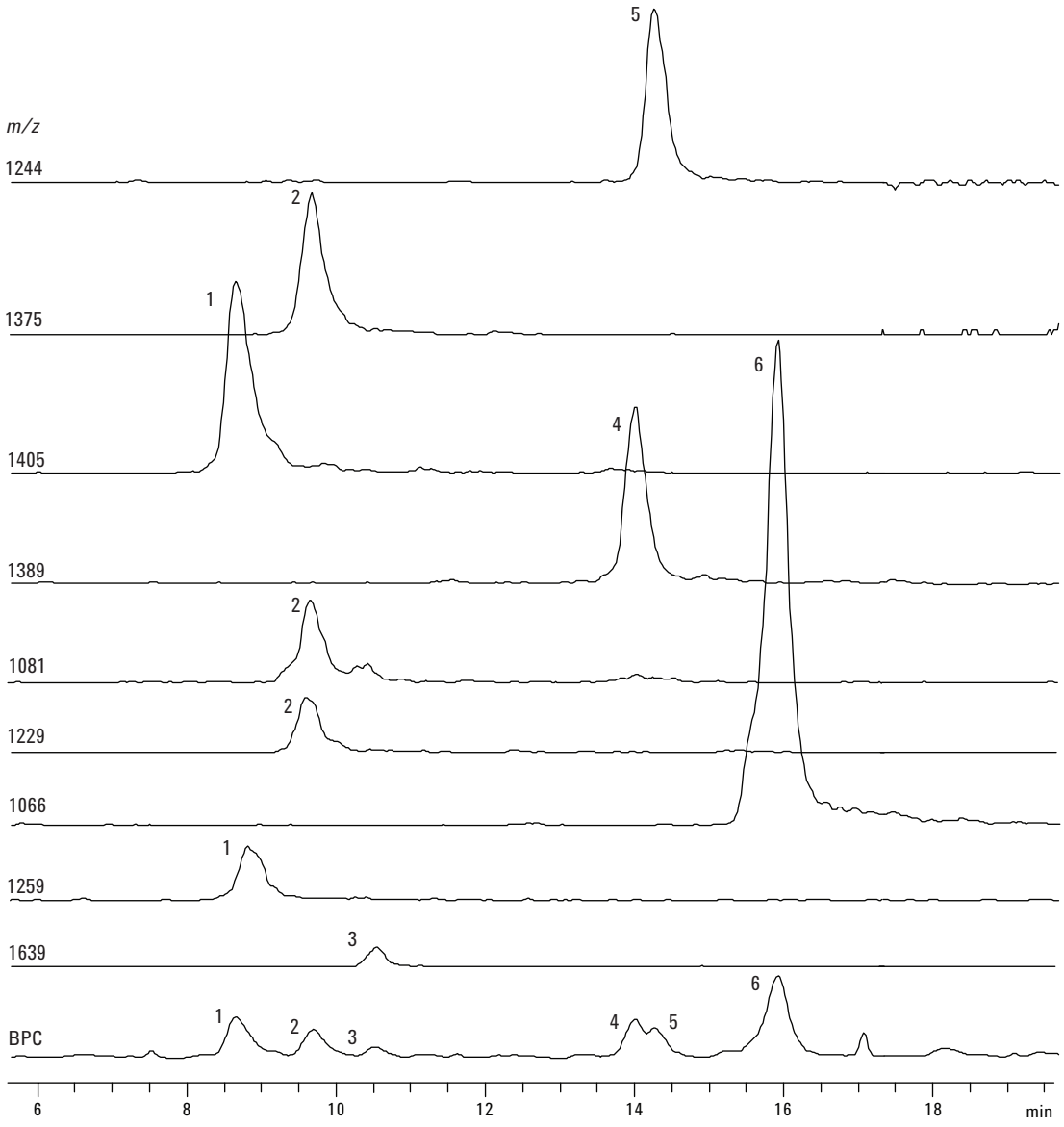


Figure 5. Stacked extracted ion profiles for the selected peaks; fragmentor at 60 V.

Results

- Negative ion spectra of these glycosylated flavanoid compounds yielded intense pseudo-molecular ions.
- The flavanoids present in the Hostas variety of plants are in the form of a series of extensively glycosylated compounds.
- The addition of TEA post-column in the mobile phase aids the formation of negative ions from heavily glycosylated species in natural products.

Conclusions

- Negative ion ESI spectra of naturally occurring compounds can yield important information about both the extent of glycosylation and the types of sugars present on the molecule
- ESI-MS under these conditions confirms the complexity of these extracts

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