Application Note: 312

Structural Determination of Flavonoids Using MS^n

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Key Words

- LCQ™
- Flavonoids
- MS^n
- Natural Products
- Structural Eludcidation

Introduction

For more than five thousand years, tea has been used in China as an herbal remedy. In recent years pharmacological studies have supported some of these health claims. Flavonoids, a class of polyphenolic compounds found in tea, plants and many fruits, have shown some promising results related to anti-cancer as well as anti-inflammatory and anti-allergy properties. For these reasons, there has been renewed interest in both detecting and characterizing these compounds.

In many instances, flavonoids exist as their glycosylated conjugate. In a typical MS/MS experiment, glycosylated moieties may be cleaved from the molecular precursor with a resulting MS/MS spectrum which is difficult to relate back to a hypothetical structure. The primary reason for the difficulty is the fact that first generation fragment ions may undergo further fragmentation, which can result in a very complex spectrum. Third and fourth generation product ions can not readily be distinguished from second generation product ions.

The Thermo Scientific LCQ Series of mass spectrometers can perform MS^n, so precursor ions are isolated before a subsequent MS/MS experiment is performed. Due to this isolation step, fragmentation spectra are generally less complex and ambiguous than traditional MS/MS spectra. Through successive MS/MS steps, product origins can be assigned. As the following experiments will show, the LCQ may "remove" glycosylated side chains in a controlled, step-by-step manner using the power of MS^n. Eventually, a core structure is exposed. A final fragmentation spectrum of the core structure yields a classic fingerprint which may then be referenced against a library MS/MS spectrum of the base component. In this way, a compound may be unequivocally classified.

Goal

This experiment demonstrates:

- The utility of MS^n for structural determination and unambiguous classification of a suspected flavonoid compound.
- The utility of MS^n for the unambiguous determination of product origin.

Experimental Conditions

Mass spectrometer: LCQ Series ion trap mass spectrometer fitted with ESI probe
Infusion: 3 µL/min using integrated syringe pump
Capillary temp: 150°C
Needle voltage: +4.2 kV
Sheath gas: 45 units
Auxiliary gas flow: 10 units

Results

Apiginin (Figure 1) (mw 270.2) is a base component of a class of flavonoids. The MS/MS spectrum of apiginin is rich in detail (Figure 2). Because the LCQ imparts energy solely onto the precursor molecule, variations in collision energy generally have very little impact on the relative ratios of the product ions. The MS/MS spectrum of apiginin will be used as our "library" spectrum or "fingerprint" for the classification of Compound "X" whose full-scan mass spectrum is shown in Figure 3.

From the spectrum in Figure 3, it is clear that compound "X" has an [M+H]+ ion at 579 and an [M+Na]+ ion at 601. The MS/MS spectrum of the [M+H]+ ion of Compound "X" (Figure 4) indicates a loss of 146 and also exhibits an ion of *m/z* 271—the *m/z* of the [M+H]+ ion of apiginin. The loss of 146 is often indicative of the loss of a deoxy-hexose sugar and the *m/z* 271.1 ion is indicative of the base component: apiginin.

At this point it is possible to do MS^3 on the 271.1 ion and compare the spectrum with that of the MS/MS spectrum of apiginin. Before we do this, however, we shall first endeavor to determine whether the *m/z* 271 ion is related to the *m/z* 433 ion. To accomplish this, an MS^3 experiment was performed on the *m/z* 433 ion and the spectrum is illustrated in Figure 5. The hypothesis that the *m/z* 271 originated from the *m/z* 433 ion has been confirmed. The difference in the two masses (162 amu) is also indicative of the loss of a hexose sugar.

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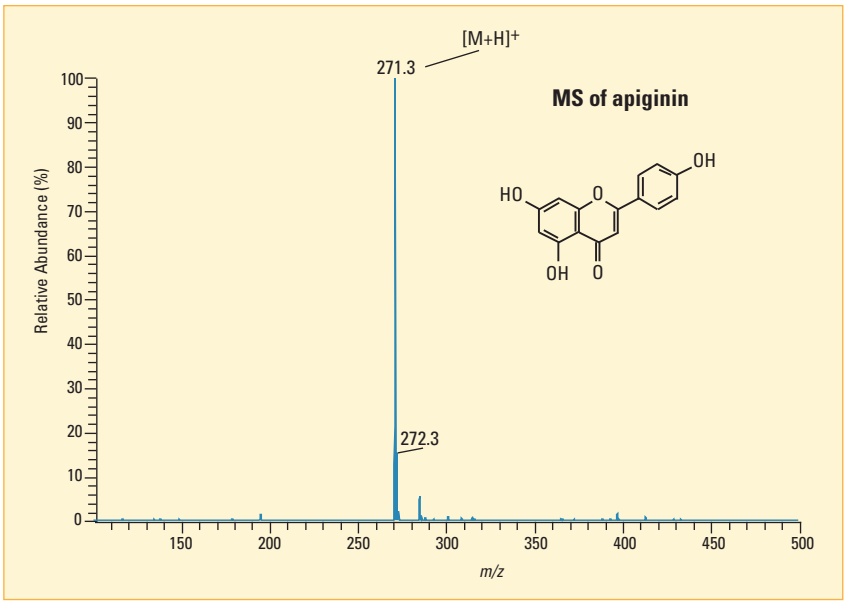

Figure 1: Structure and full-scan mass spectrum of apiginin

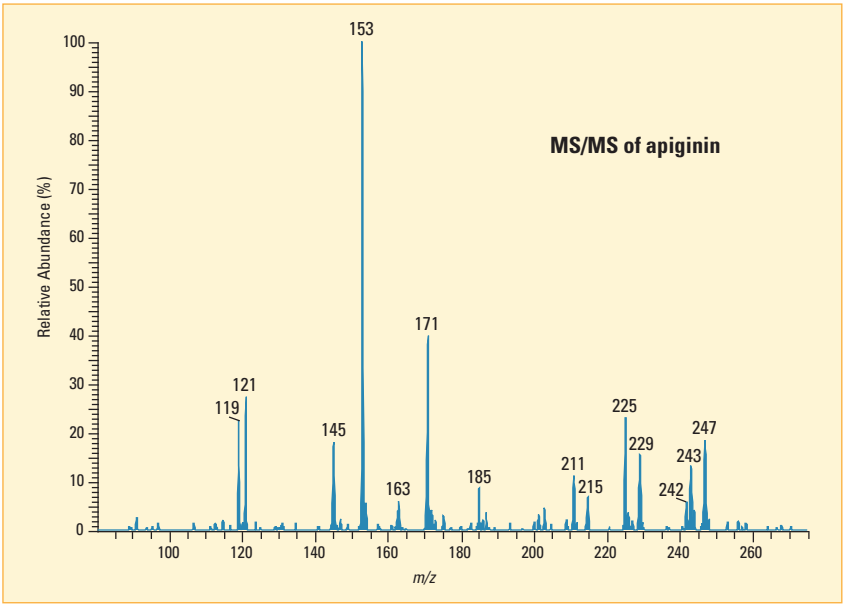

Figure 2: Full-scan MS/MS (*m/z* 271) spectrum of apiginin

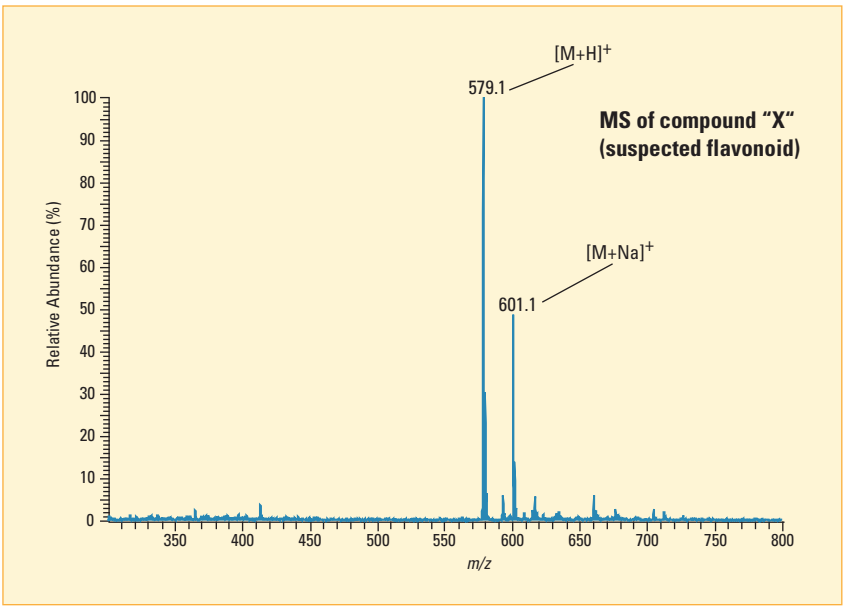

Figure 3: Full-scan mass spectrum of Compound "X"

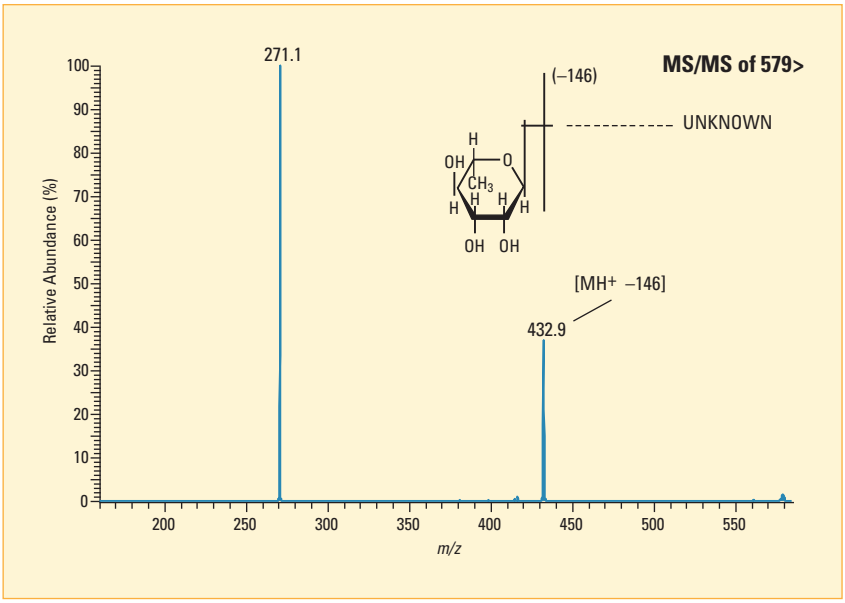

Figure 4: Full-scan MS/MS (*m/z* 579) mass spectrum of Compound "X".

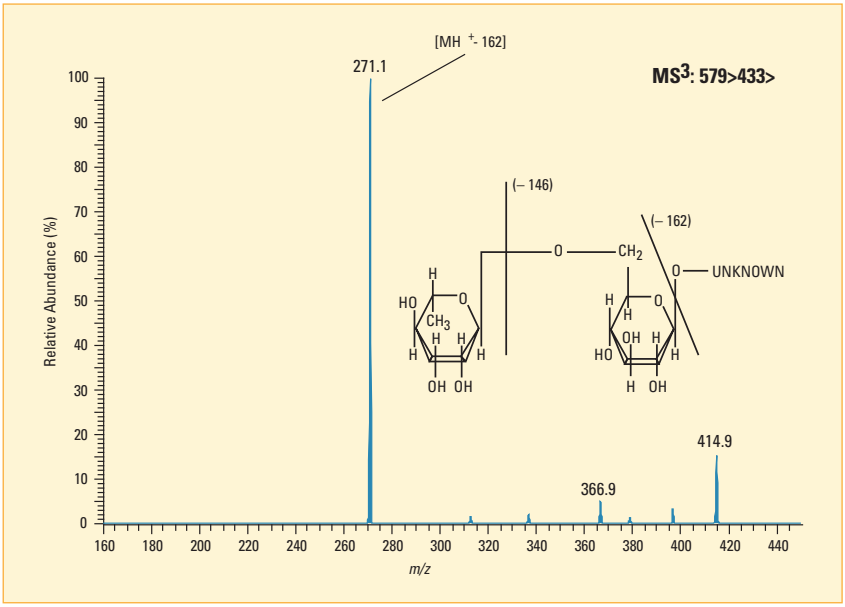

Figure 5: Full-scan MS^3 (579>433>) mass spectrum of Compound "X"

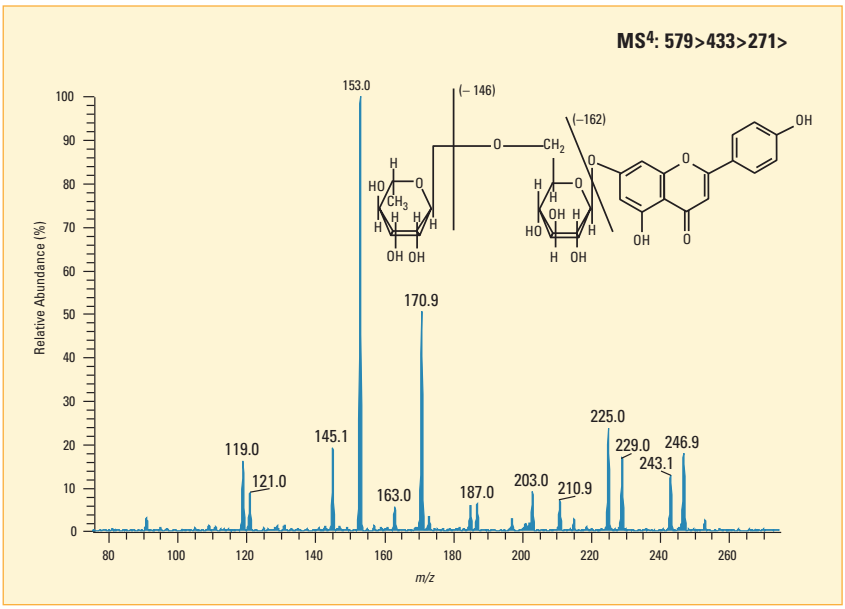

Figure 6: Full-scan MS^4 (579>433>271>) mass spectrum of Compound "X"

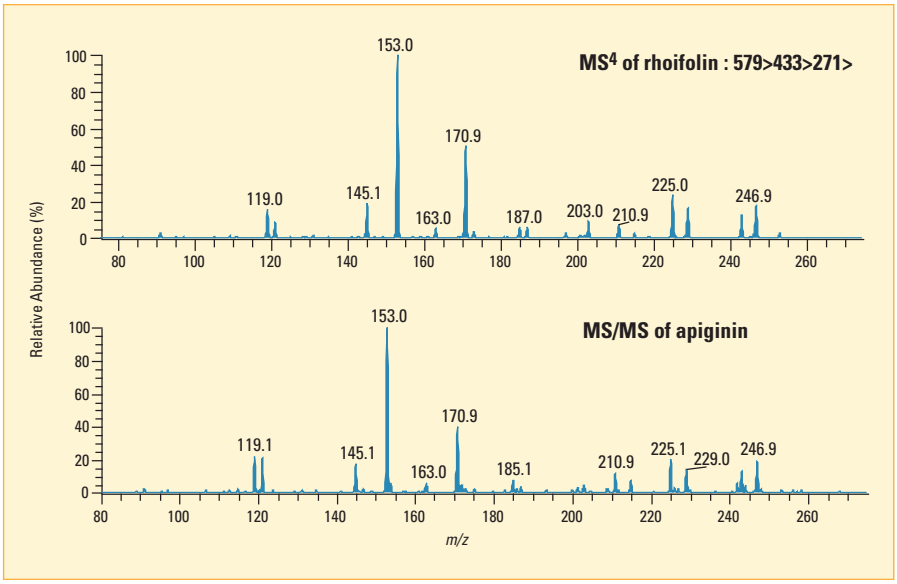

Figure 7: Comparison of full-scan MS/MS (271) Spectrum of apiginin with the full-scan MS^4 (579>433>271) mass spectrum of Compound "X"

Up to now, the only evidence that Compound "X" belongs to the flavonoid class is the presence of the *m/z* 271 ion. This is far from compelling evidence. Compelling evidence would be achieved if the MS/MS of this *m/z* 271 ion yields a spectrum which is similar to the MS/MS spectrum of apiginin. This hypothesis is tested via the MS^4 experiment of the *m/z* 271 ion. The spectrum (Figure 6) is an exact match of the spectrum generated by the MS/MS experiment of apiginin (Figure 7).

Compound "X" is a flavonoid. In fact, it is the flavonoid rhoifolin (apiginin 7-0-neohesperidoside).

Conclusions

Electrospray ionization of an unknown compound provides a soft ionization technique allowing the generation of an intact pseudomolecular ion at *m/z* 579. The MS/MS experiment yielded two major ions which were indicative of the loss of a deoxy-hexose sugar (*m/z* 433) and a product mass which was the same as a base component of flavonoids (*m/z* 271). MS^3 of the *m/z* 433 ion proved that the *m/z* 271 ion was a product of the *m/z* 433 ion and also indicated the loss of a hexose sugar. The MS^4 experiment on the *m/z* 271 ion resulted in a fragmentation spectrum which was a direct match with the fragment spectrum of apiginin. The direct match indicates that the unknown compound belongs to the flavonoid class.

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