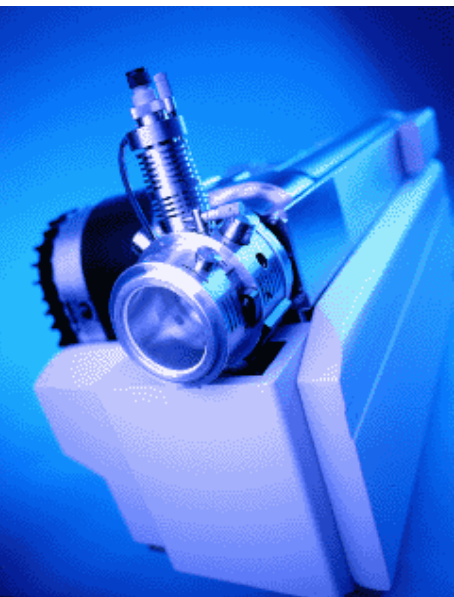




Purdue-UAB Botanicals Center for Age-Related Disease

MS/MS analysis of Polyphenols

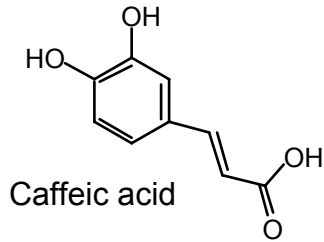


**Jeevan Prasain Ph.D.
Pharmacology & Toxicology
UAB**

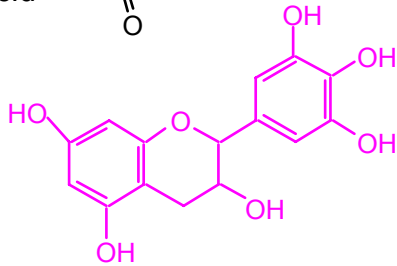


Polyphenols

Phenolic acids
and derivatives



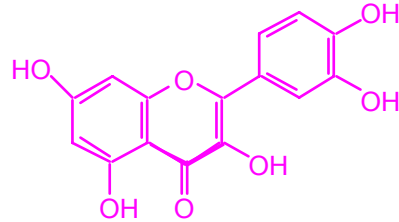
EGC
(Flavanol)



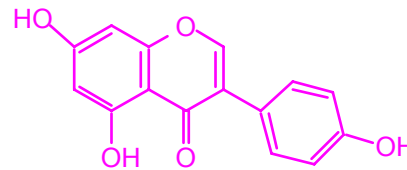
Flavonoids

Flavanols Flavonols Isoflavones

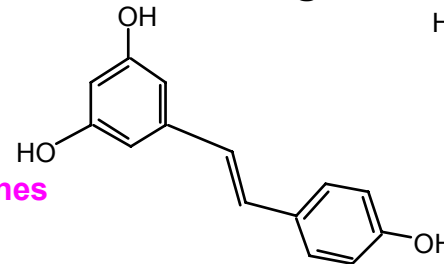
Quercetin
(Flavonol)



Genistein (Isoflavone)

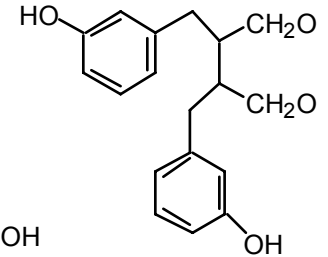


Stilbenes



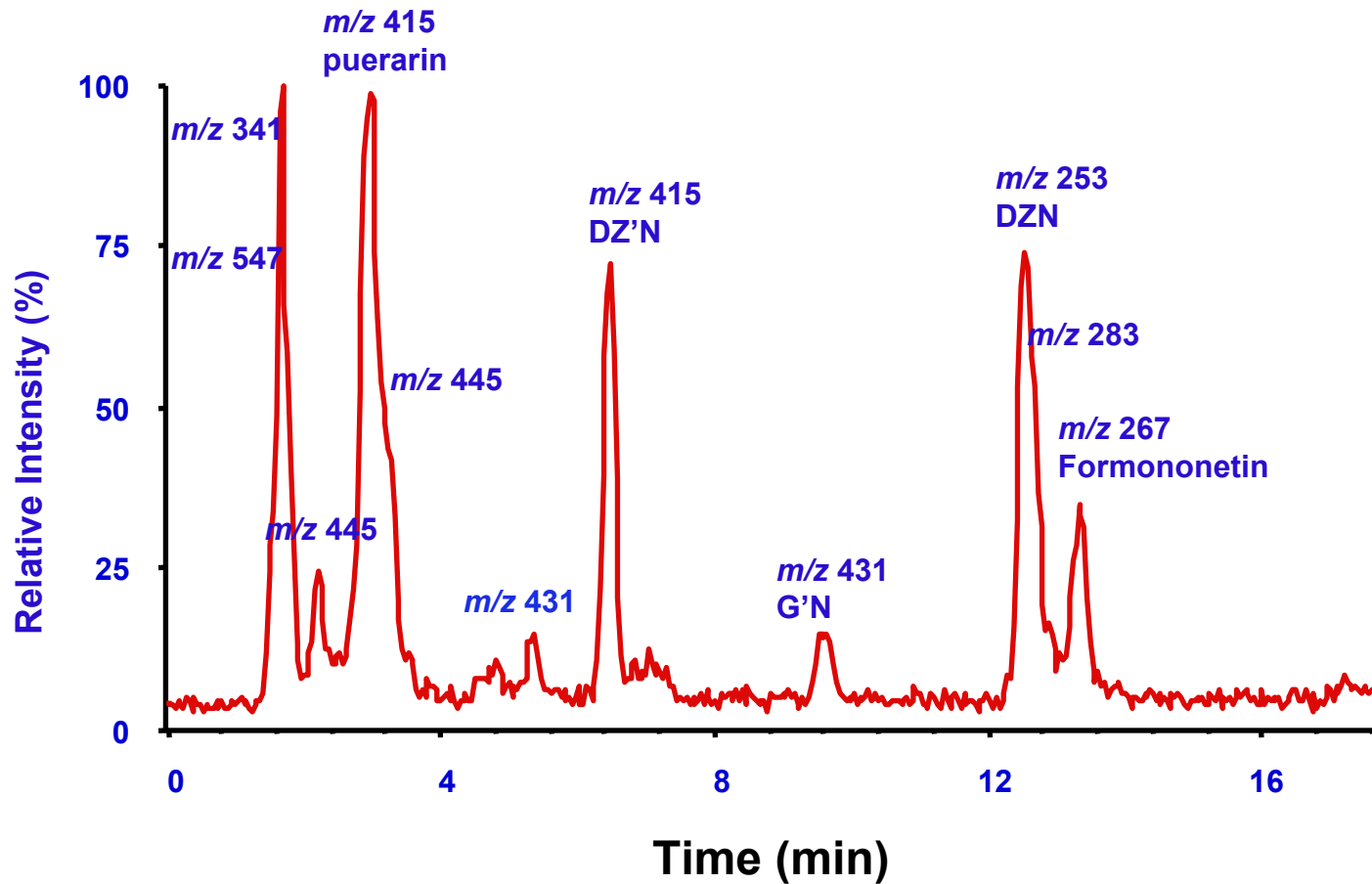
Resveratrol
(Stilbene)

Lignans



Enterodiol
(Lignan)

LC-MS Profile of the methanolic extract of KDS
Column: C₈ Aquapore; 7 μm, 100 x 4.6 mm i.d.
Solvent: CH₃CN:H₂O (10-40%, run time 30 min)



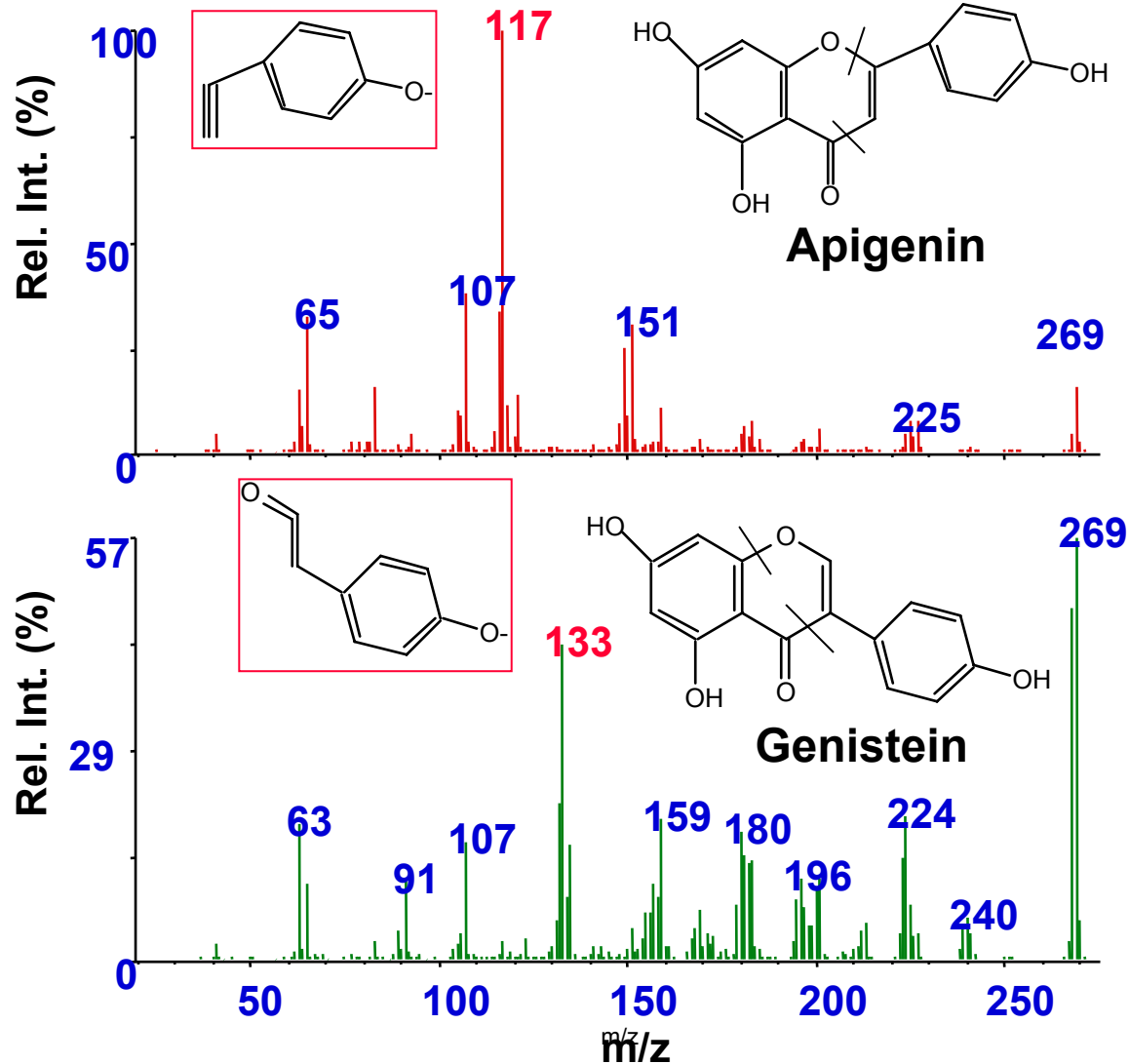
What is tandem mass spectrometry?

The ability to induce fragmentation and perform successive mass spectrometry experiments (MS/MS) on those fragments. In MS/MS mode, product ion, precursor ion and constant neutral loss scans are performed. Multiple reaction monitoring (MRM) is useful technique for quantitation.

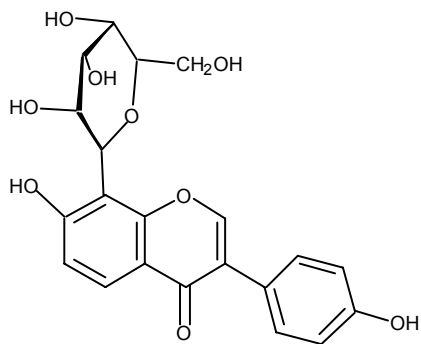
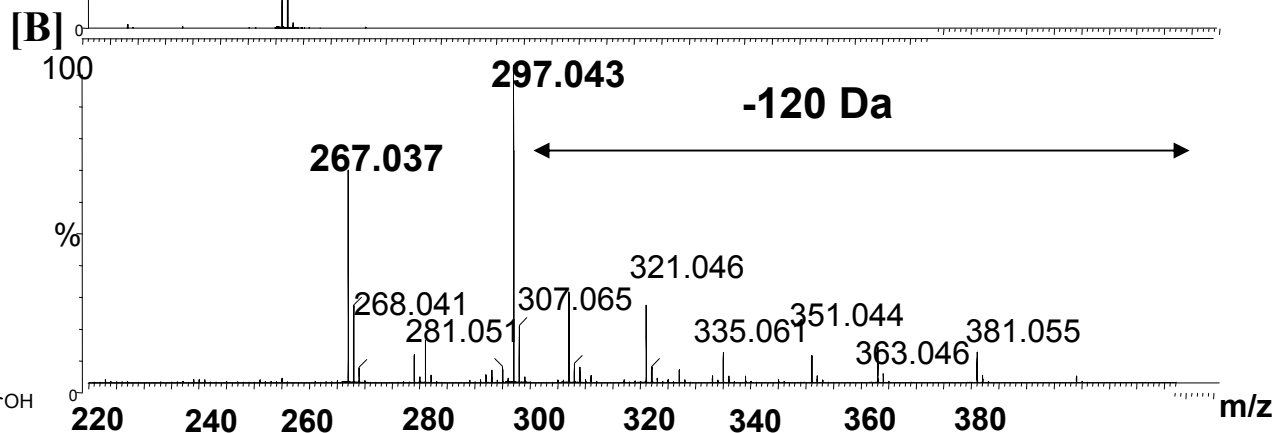
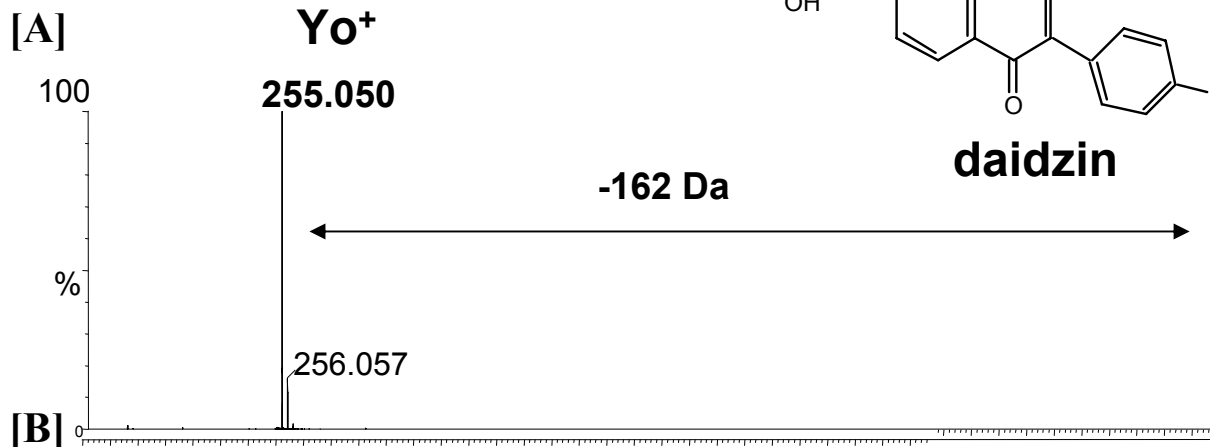
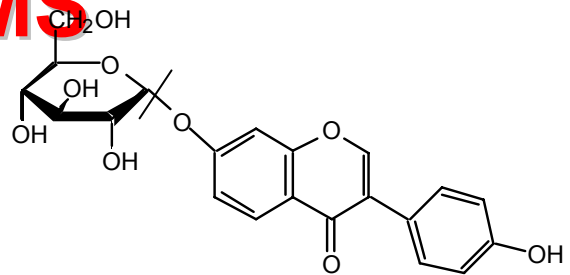
MS/MS data interpretation

- 1. Identification of molecular ions or quasi-molecular ions.**
- 2. Origin of product ions.**
- 3. Stability and relative intensity of ions.**

Product ion spectra apigenin vs. genistein

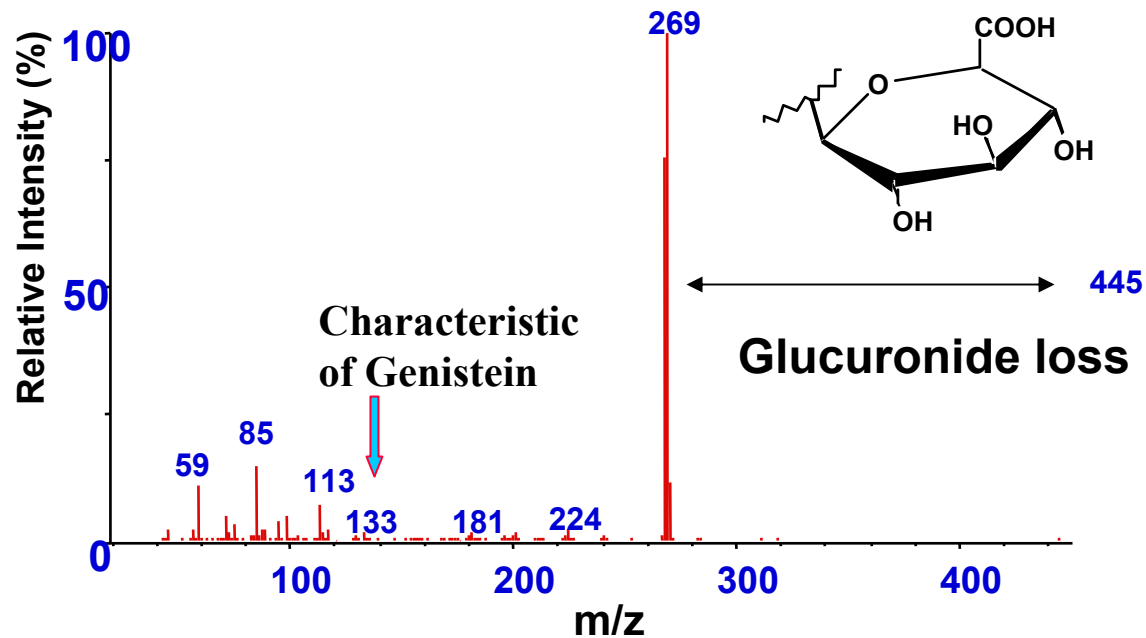


Product ion spectra of daidzin [A] and puerarin [B] in ESI-MS/MS

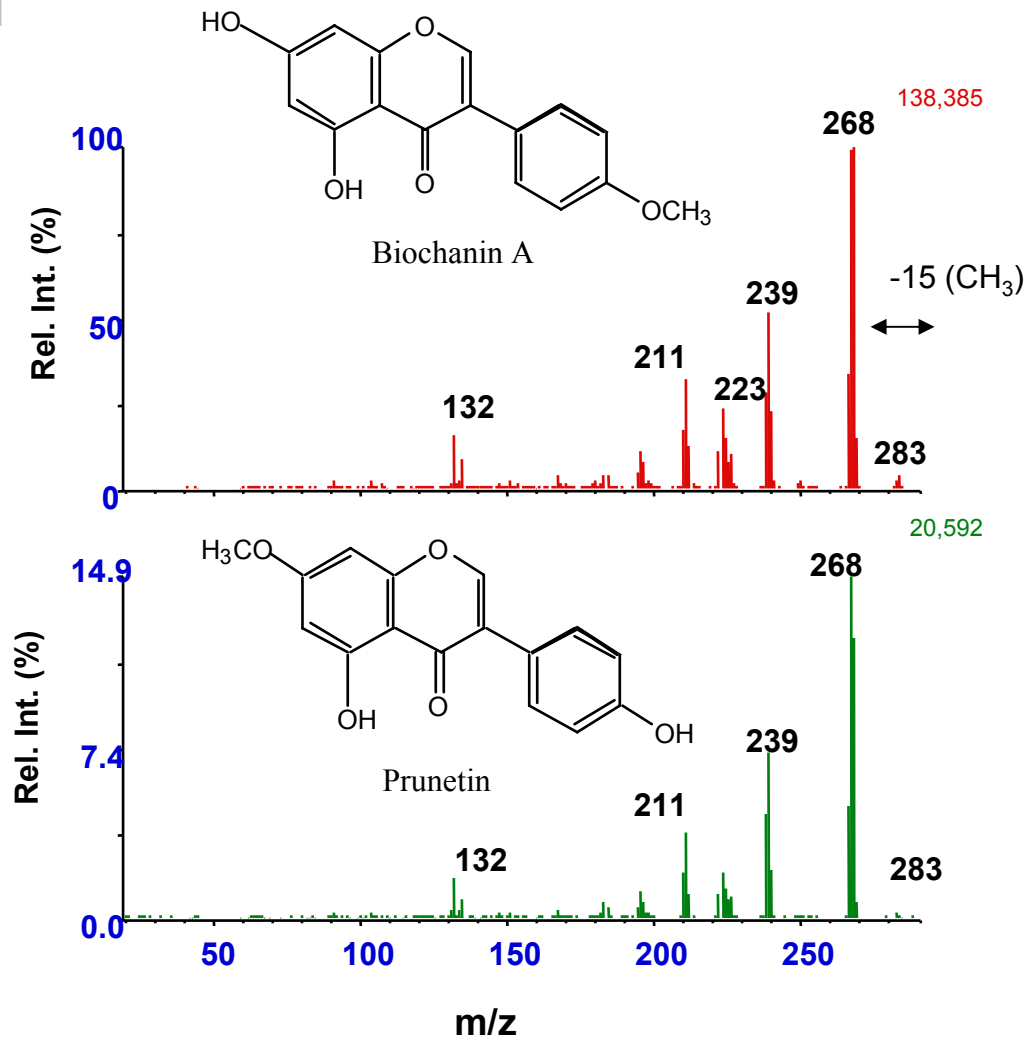


Puerarin

Product ion spectrum of genistein glucuronide in ESI-MS/MS

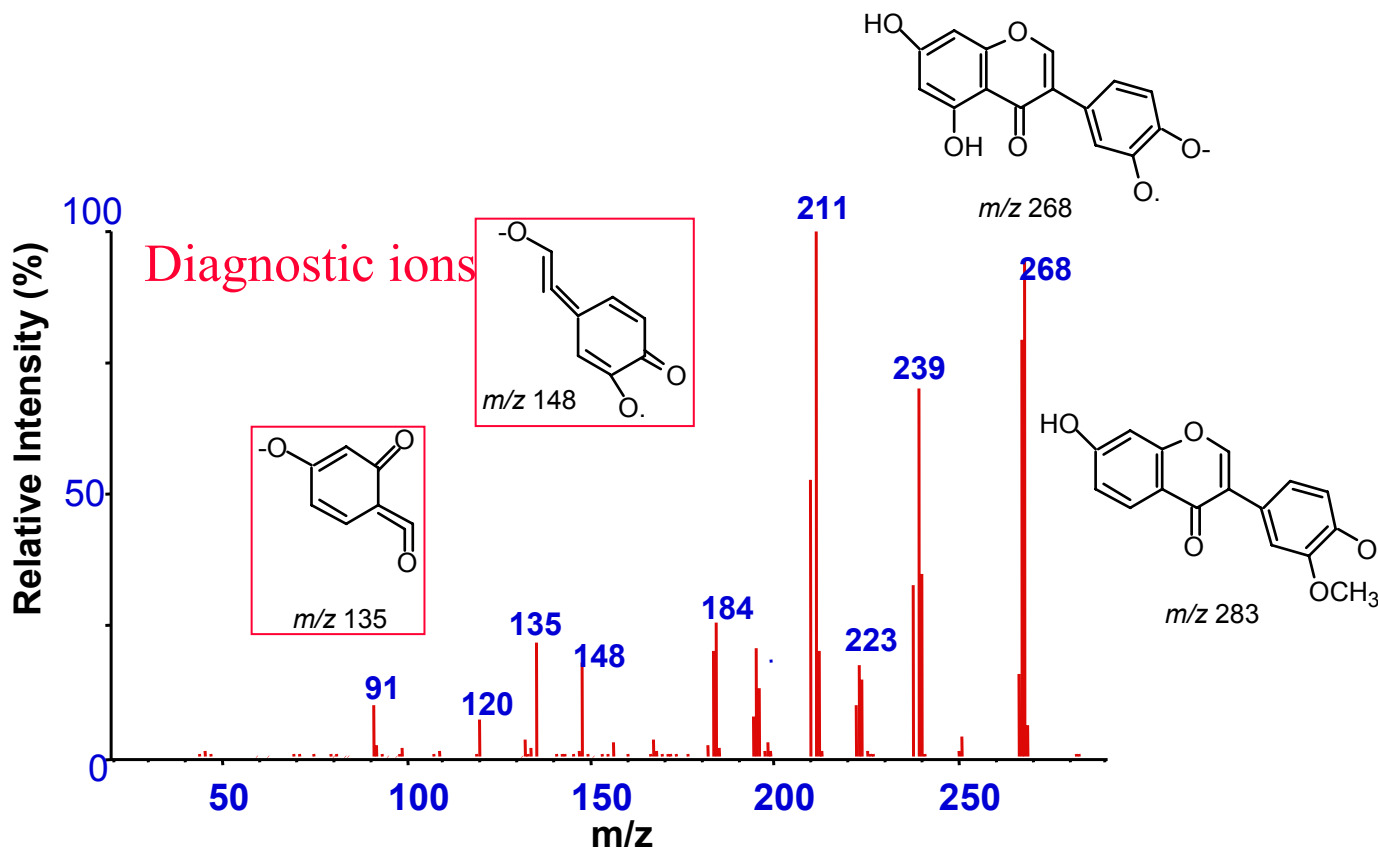


ESI-MS/MS Spectra of Biochanin A and Prunetin

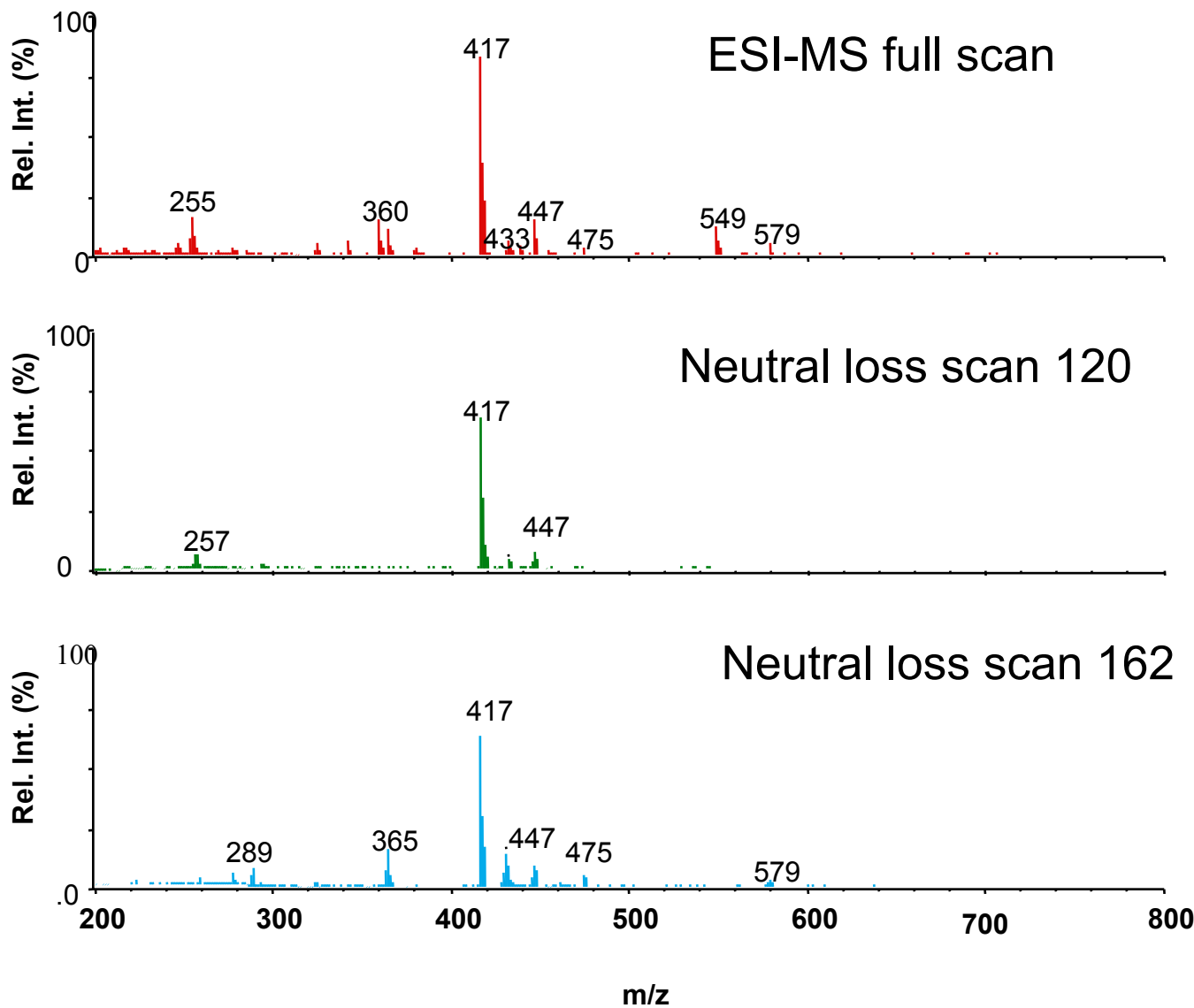


Loss of methyl radical is characteristic for methylated flavonoids

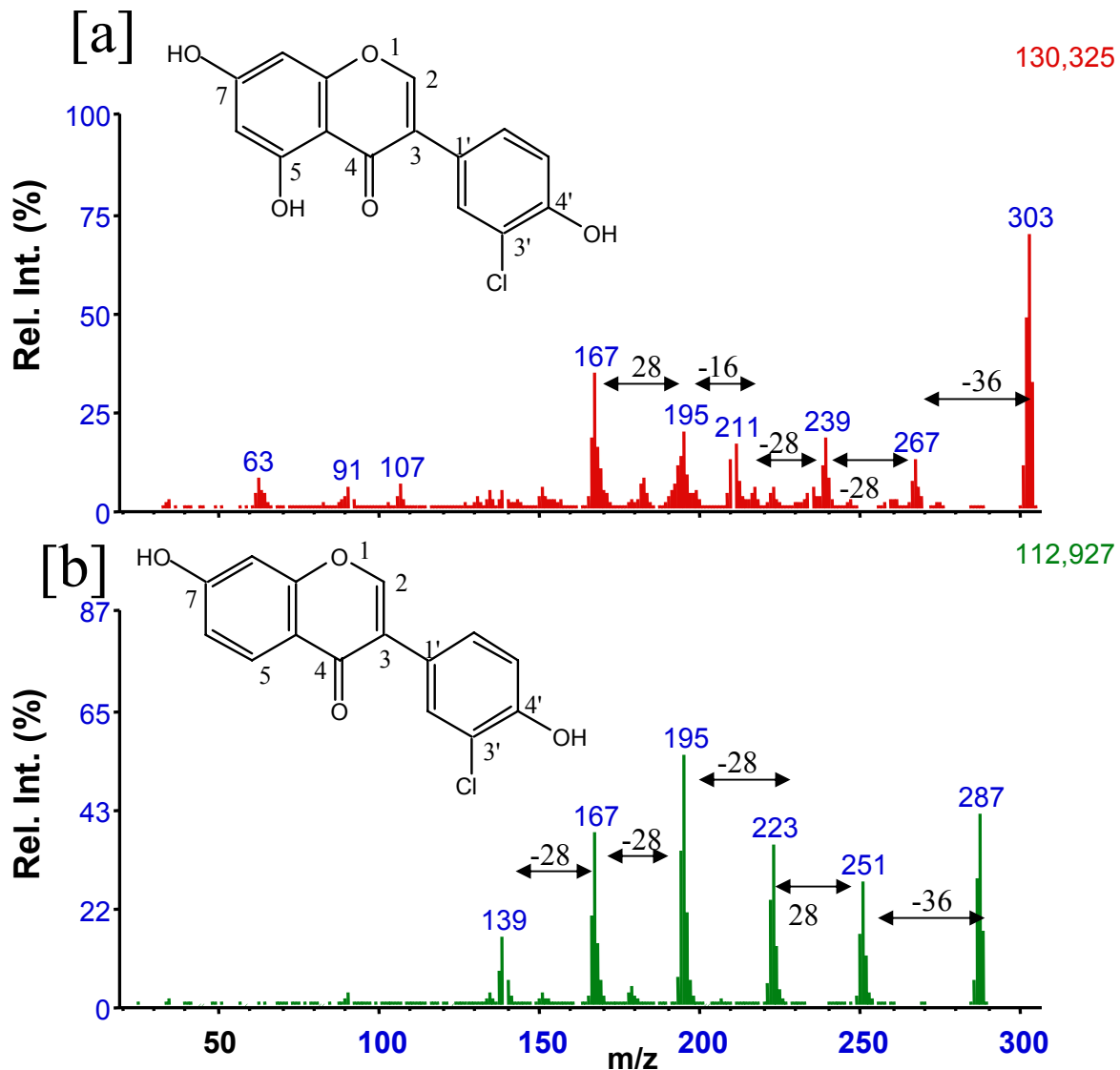
Structural identification of unknown based on product ions



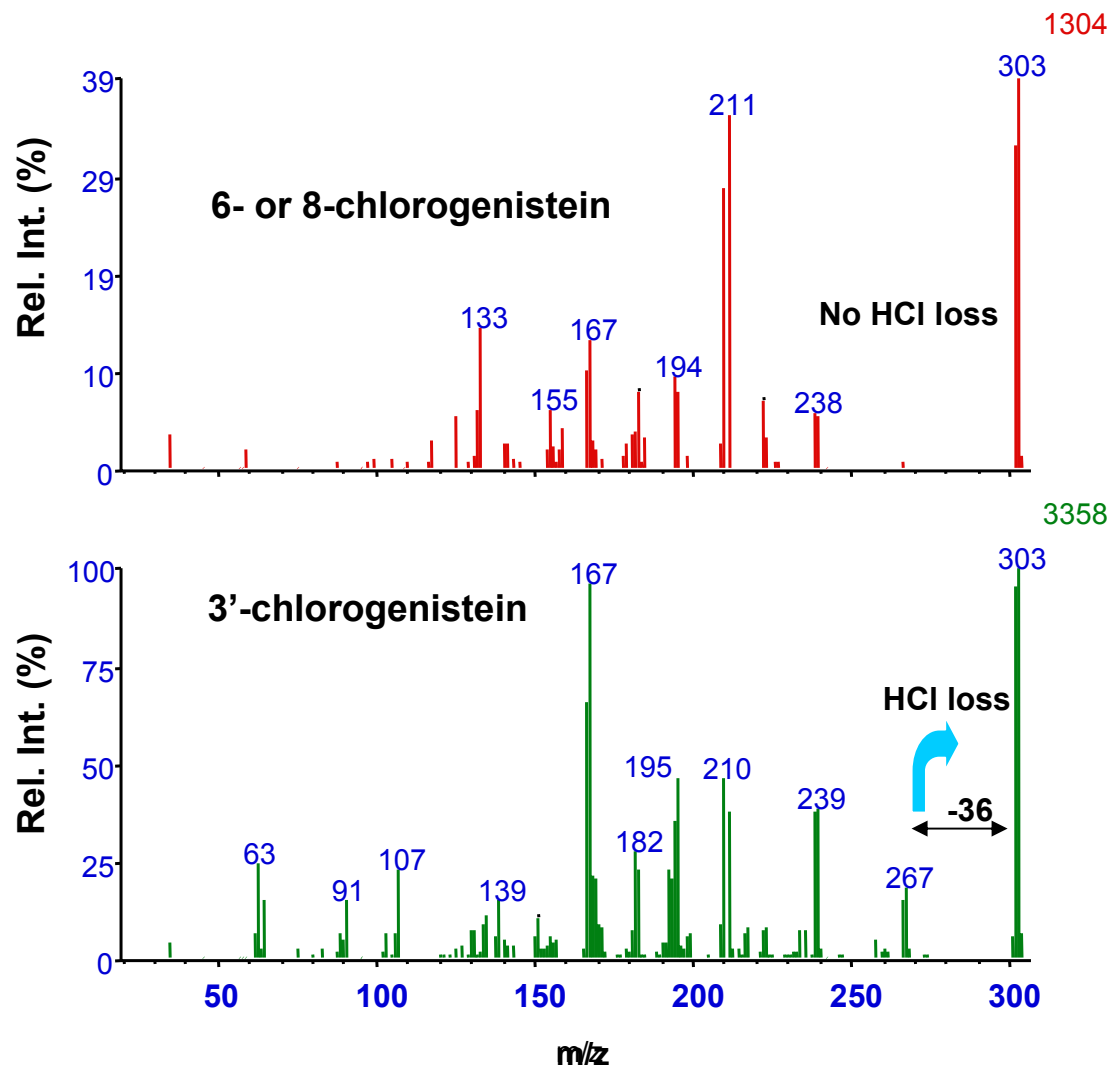
Full scan ESI-MS of the methanolic extract of kudzu dietary supplement. Constant neutral loss scans to identify the isoflavone components.



Comparison of the product ions obtained in ESI-MS/MS of 3'-chlorogenistein [a] and 3'-chlorodaidzein [b]

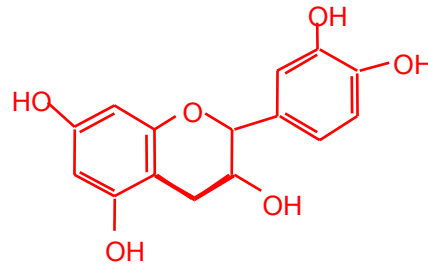


Product ion spectra of a reaction product at m/z 303 in LC-MS/MS

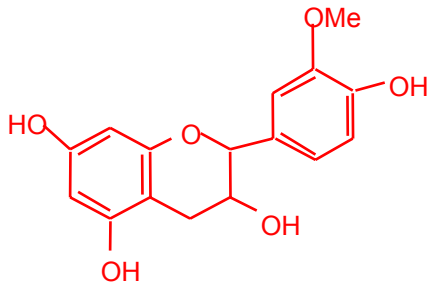


HCl loss is diagnostic for positioning chlorine in the aromatic ring.

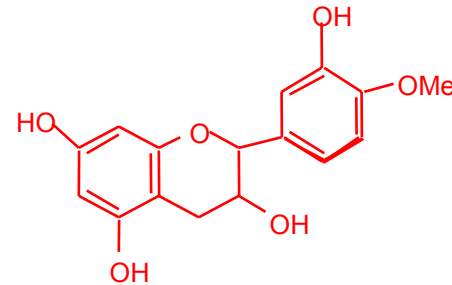
Urinary metabolites detected in a rat fed with grape seed extract



catechin

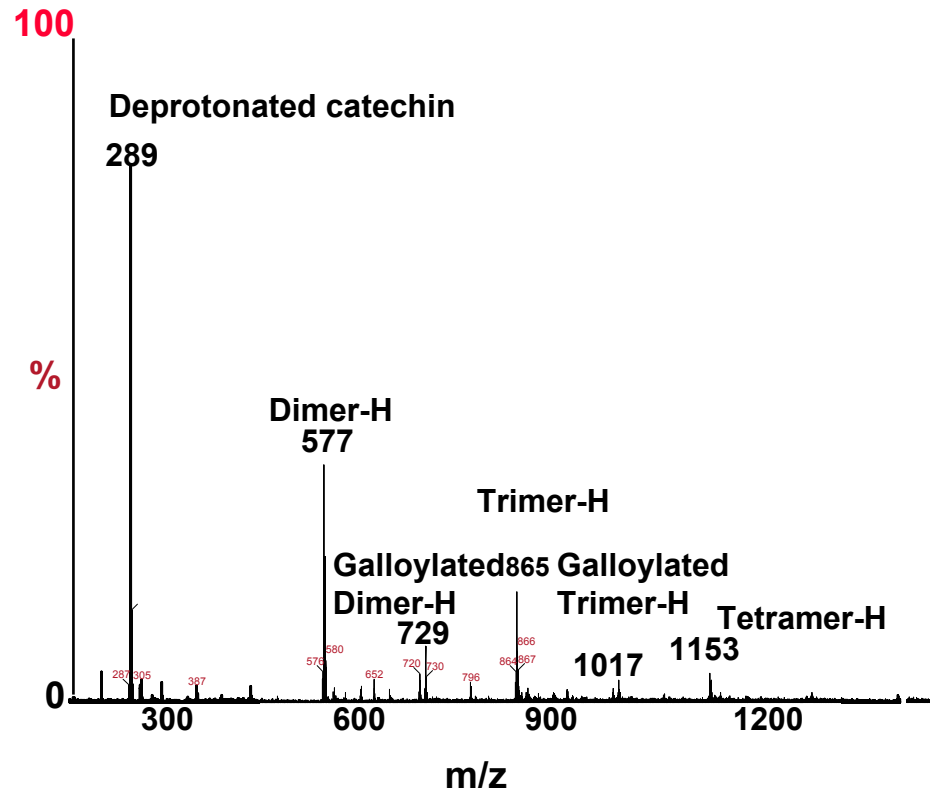


3'-O-methylcatechin

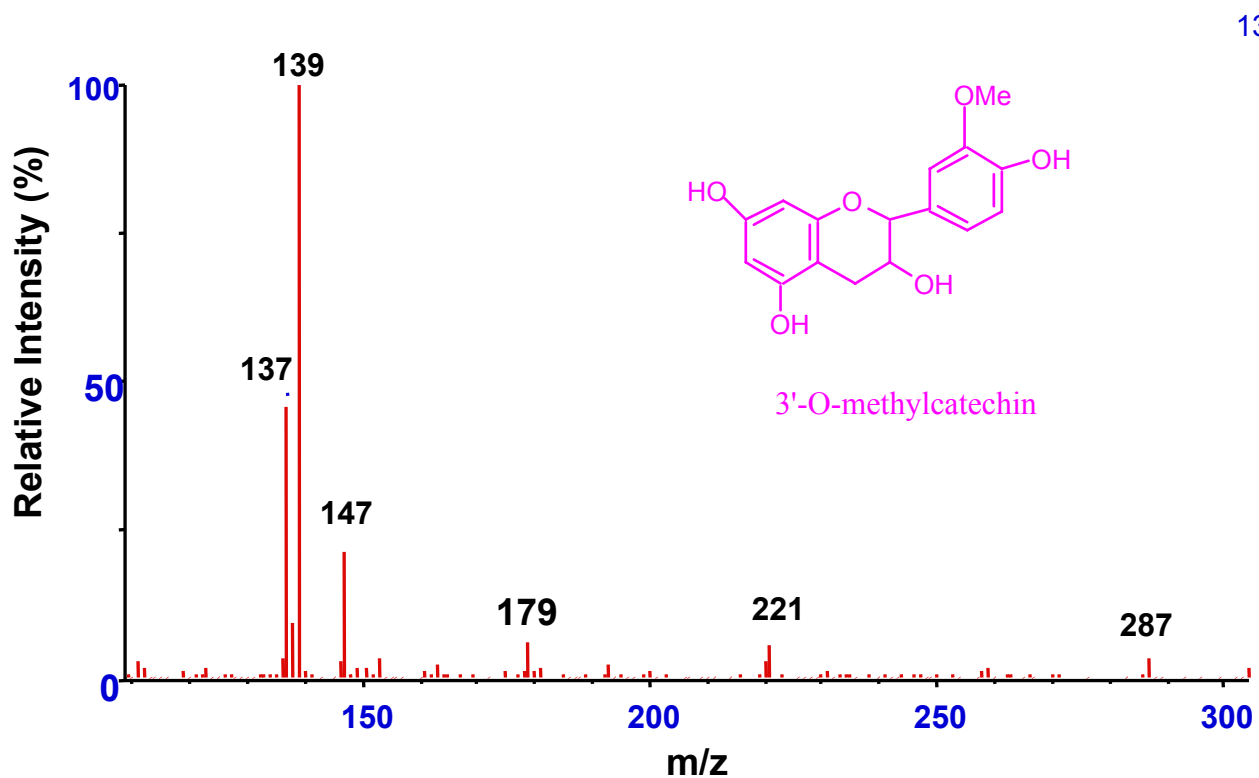


4'-O-methylcatechin

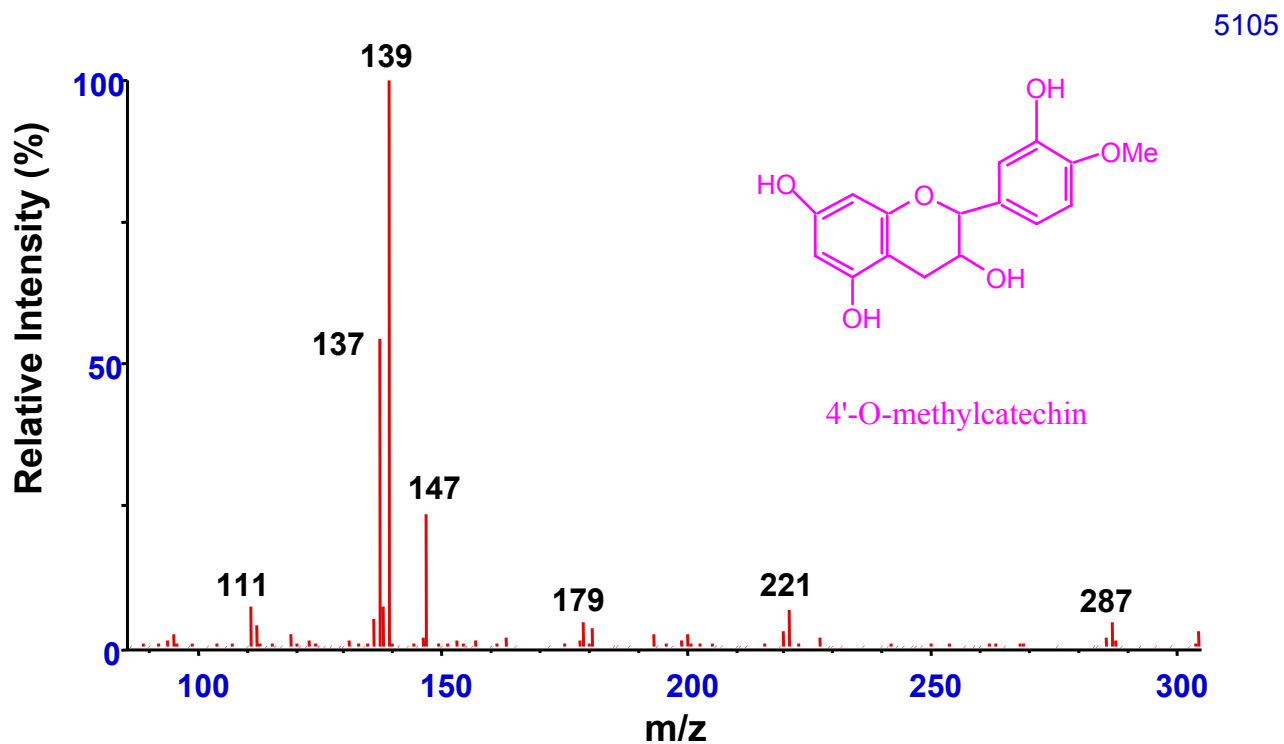
Full-scan ESI-MS spectrum of the methanolic extract of grape seed extract



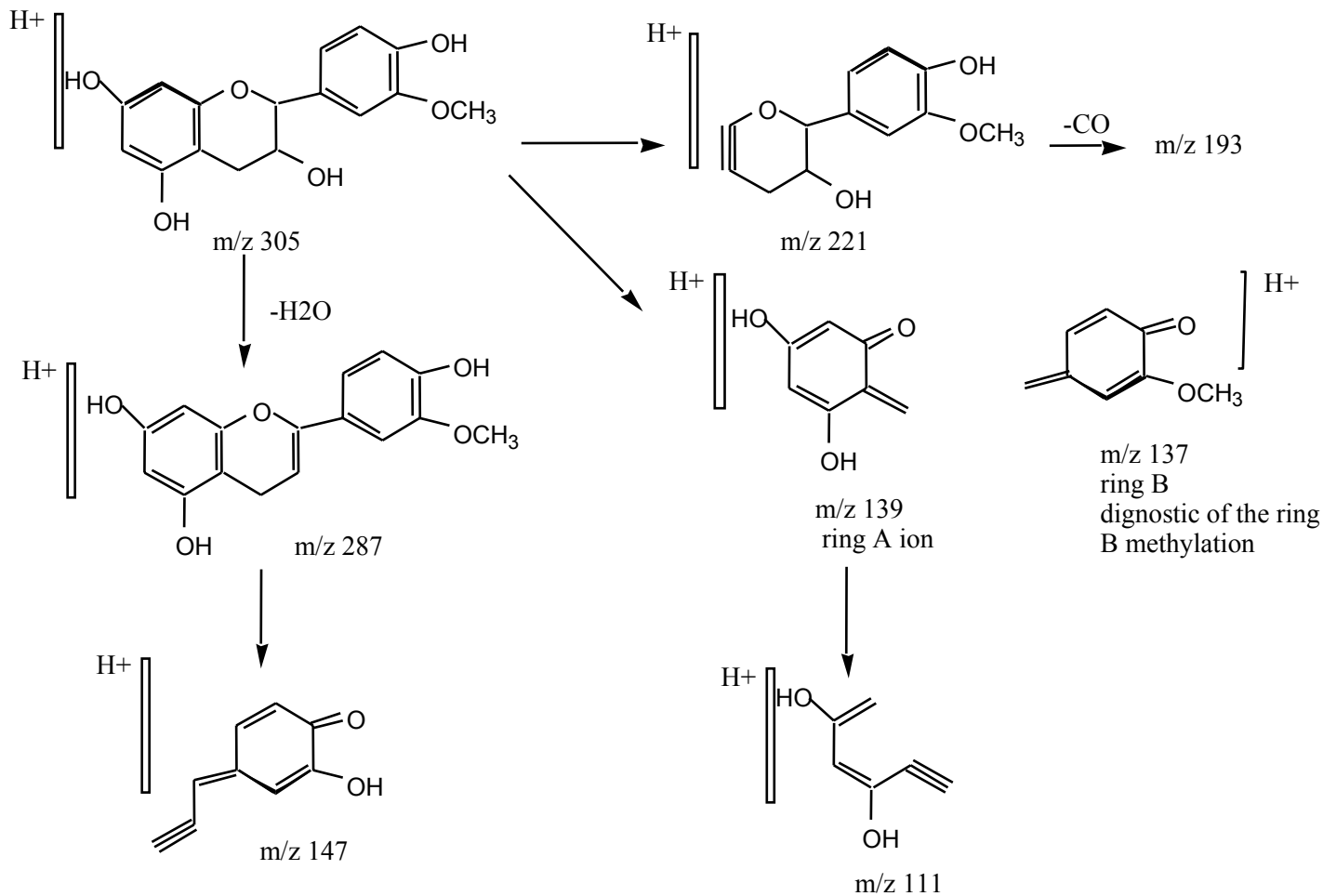
Product ion spectrum of the ion m/z 305 in LC-MS/MS analysis (Rt. 7.10 min)



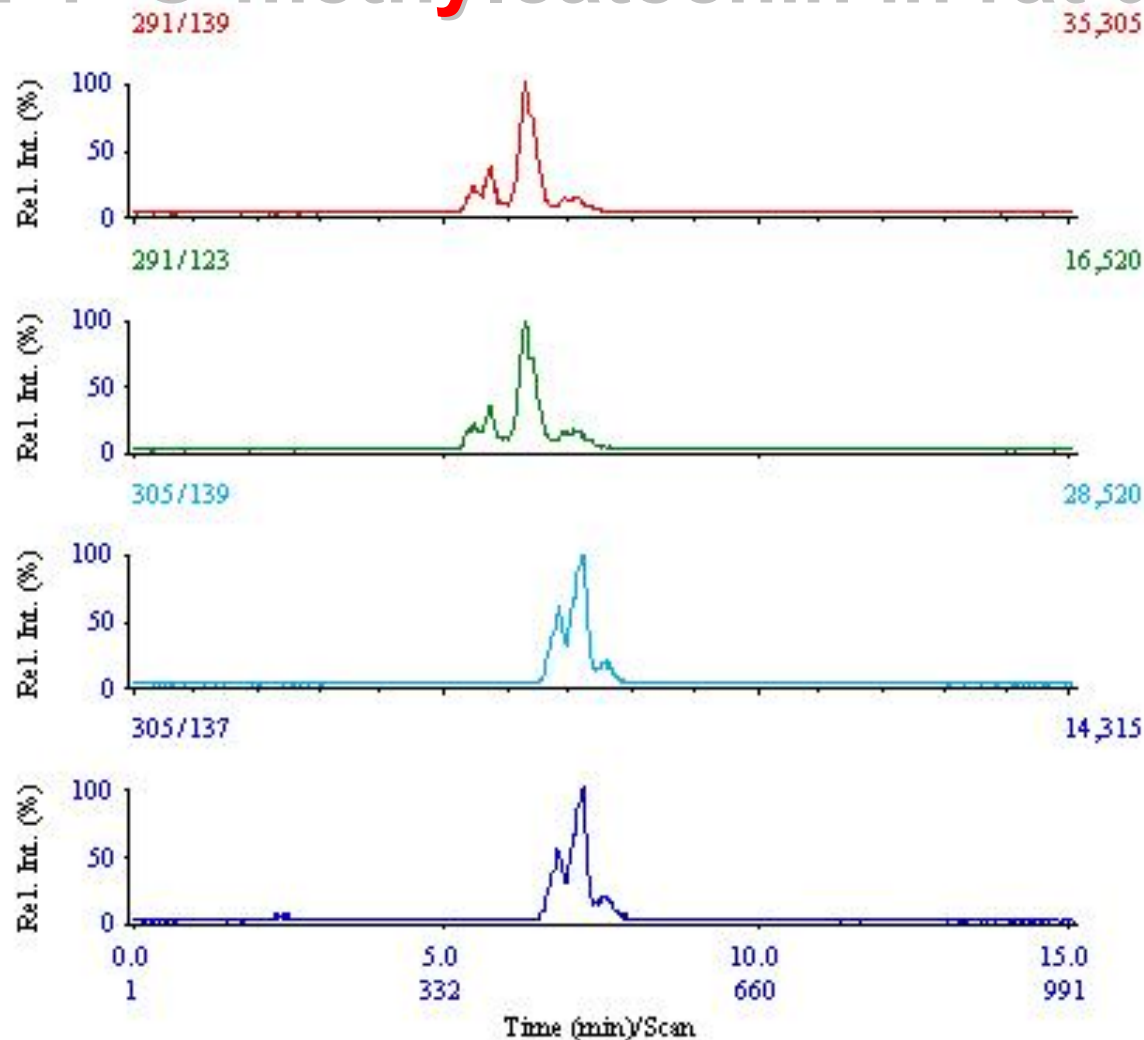
Product ion spectrum of the ion m/z 305 in LC-MS/MS analysis (Rt. 7.88 min)



Proposed structures of product ions obtained from m/z 305 in ESI-MS/MS



LC-MS/MS-MRM spectra for catechin and 3'- and 4'-O-methylcatechin in rat urine



Conclusions

- The CID of isoflavones in ESI-MS generates a series of cluster ions due to the subsequent losses of carbonyl/aldehyde or oxygen.
- The presence of prominent ions at m/z 133 and 135 in MS/MS of genistein and daidzein indicates that they are not originated from the ring A.
- Isoflavones glycosides (O- and C-glycosides) generate different product ions in ESI-MS/MS due to the neutral losses of 162 and 120, respectively.
- The major fragmentation of isoflavones with chlorinated ring B in MS/MS starts with elimination of HCl, whereas the loss from product ions was observed with those chlorine in the ring A.