Botanical workshop
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LC-MS Analysis of Botanicals

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Applications of mass spectrometry in botanical research

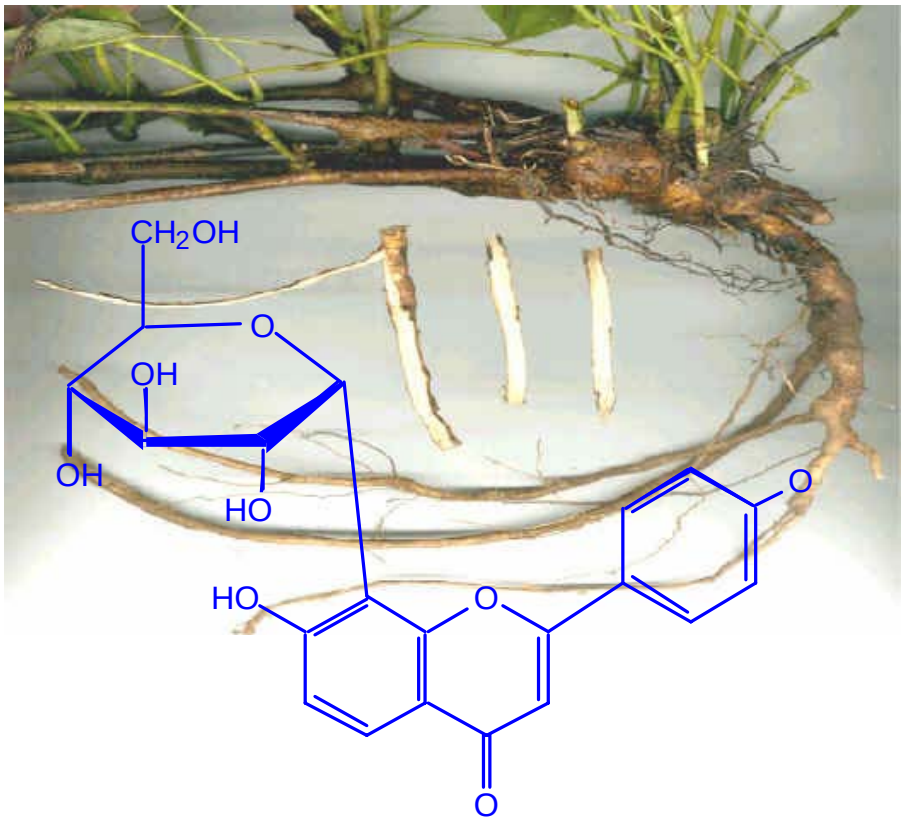
Identification, characterization and quantitation of chemical components by LC-MS and MS-MS

Purity assessment and quality control of dietary supplements/neutraceuticals

***In vitro & in vivo* metabolism, bioavailability, toxicity and pharmacokinetics of drugs and their metabolites in biological samples**

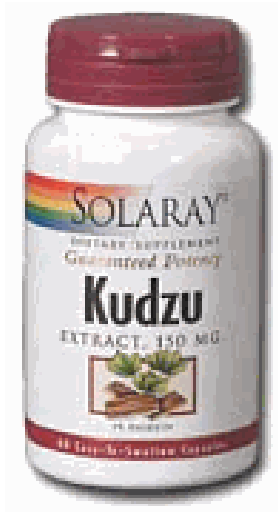
Molecular target profiling - proteomics/metabolomics and chemical imaging of analytes

Kudzu root (Radix Puerariae) as a dietary supplement

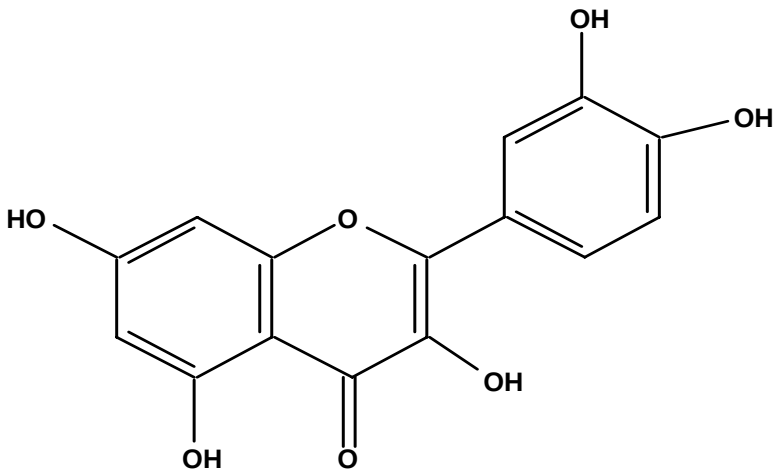


Puerarin (PN)

- Different brands are available

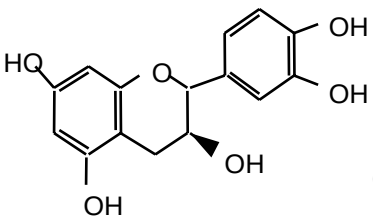


The fruit of the American cranberry is a rich source of dietary flavonoids and considered to be beneficial for Urinary Tract Infection

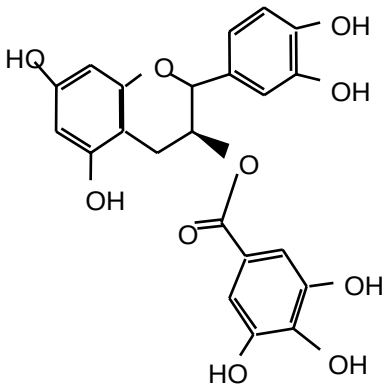


Quercetin

Grape seeds are one of the richest sources of Proanthocyanidins (B type)

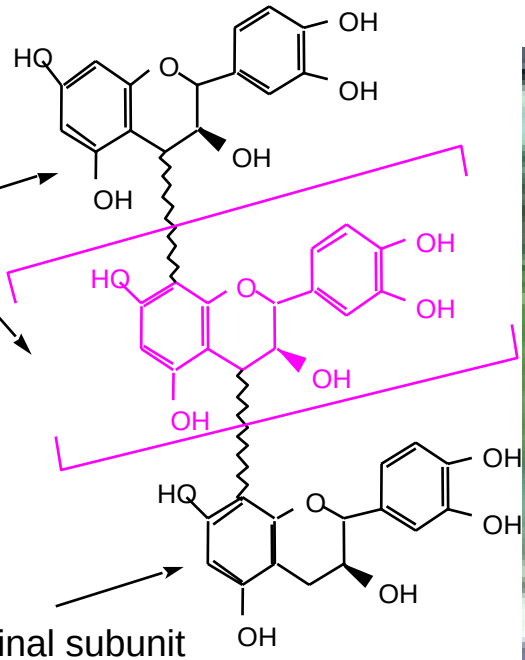


Catechin



Catechin gallate

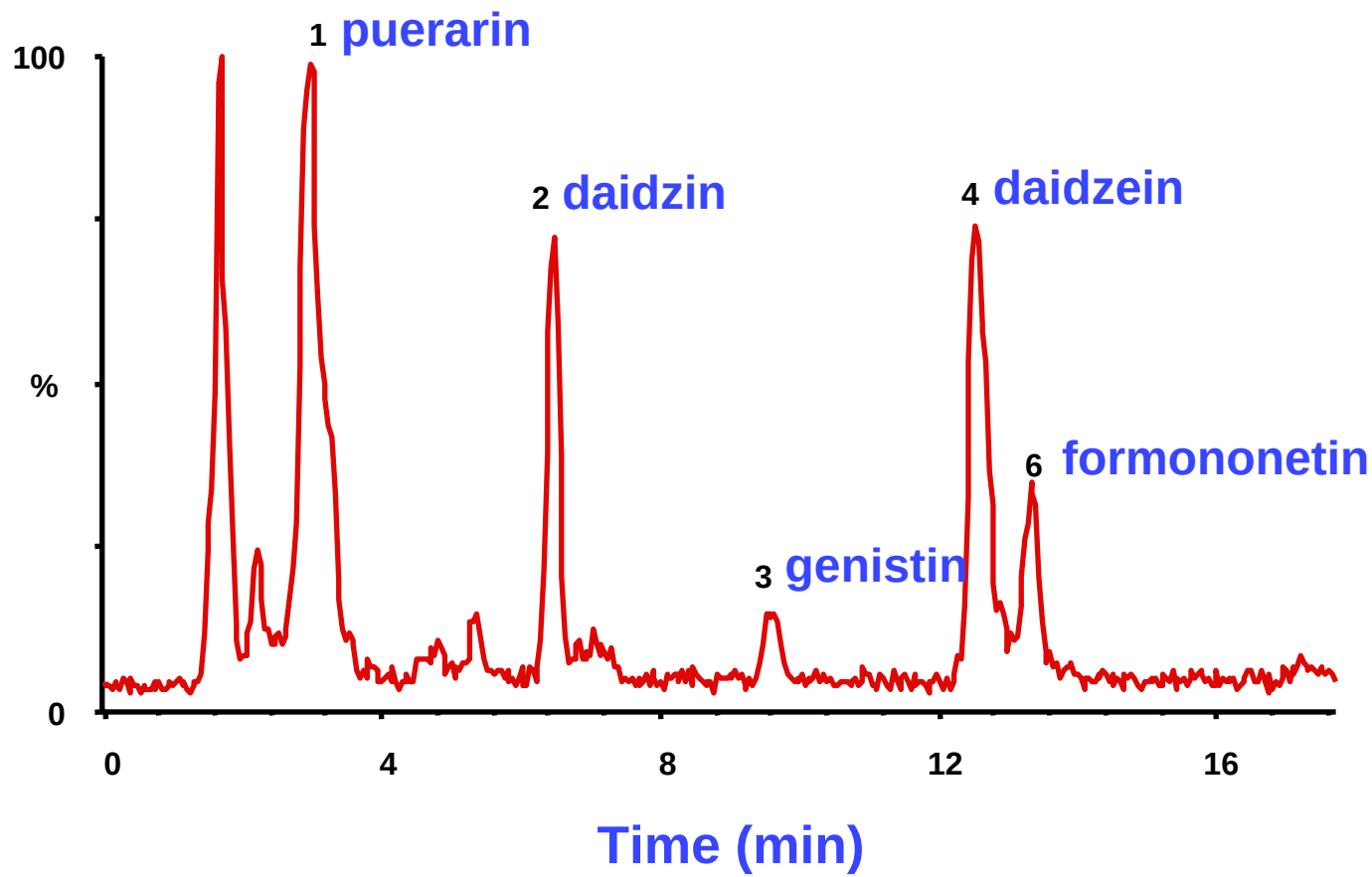
extension subunit



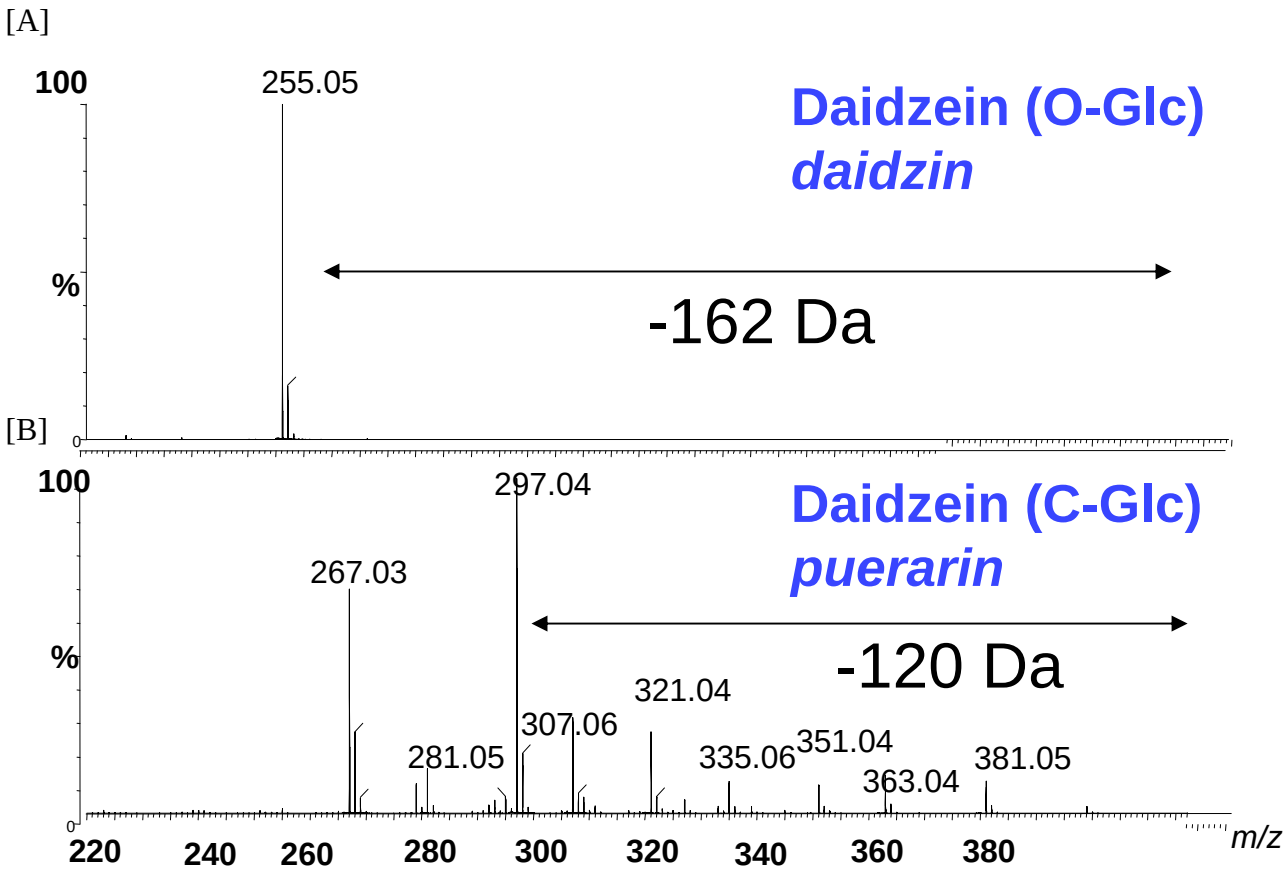
terminal subunit

Isoflavonoids in Kudzu Dietary Supplements/root culture extract

TIC of reverse-phase LC-MS of kudzu dietary supplement (KDS) isoflavones

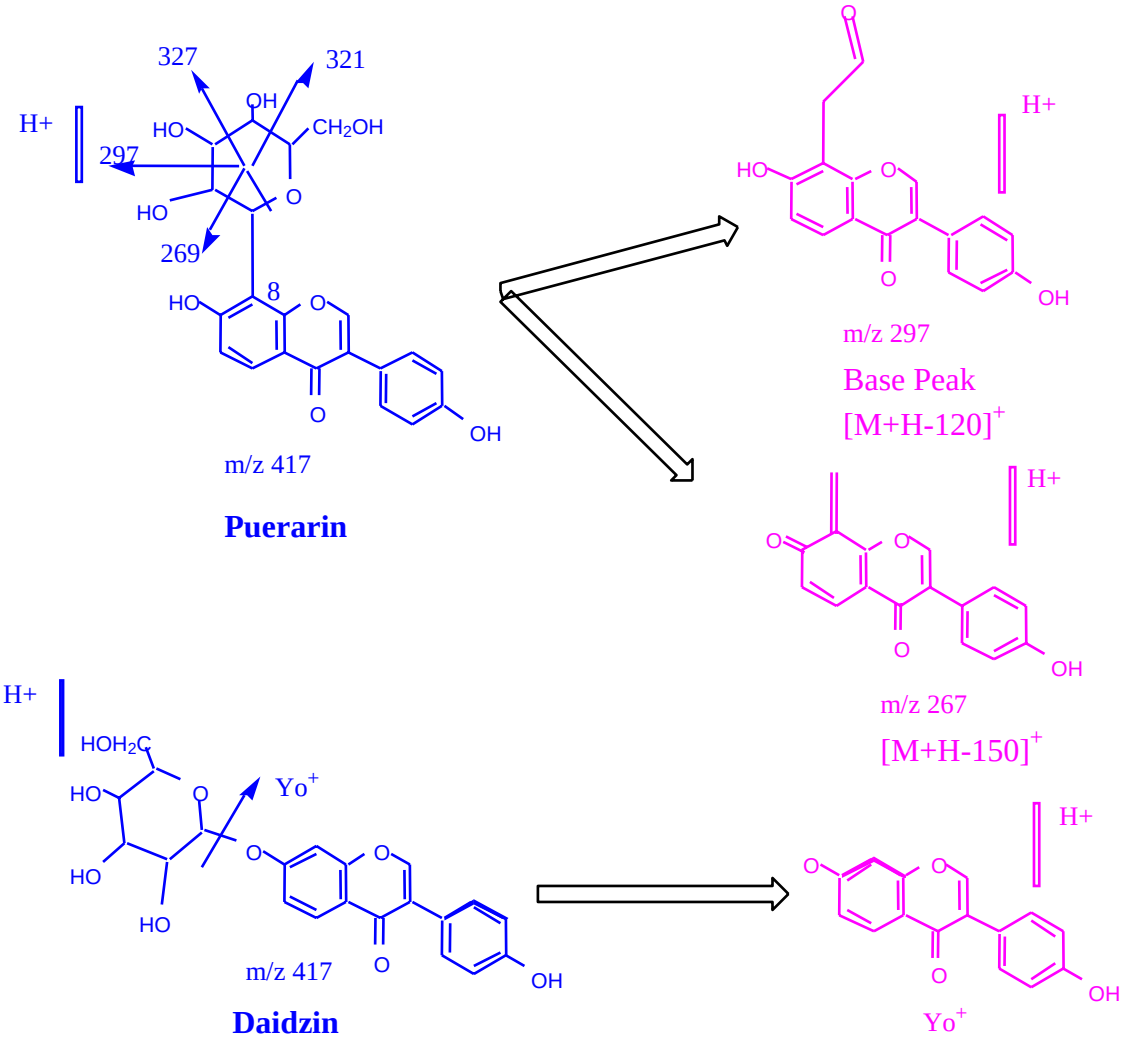


MS/MS spectra of protonated O- and C-glycosides of daidzein (m/z 417)

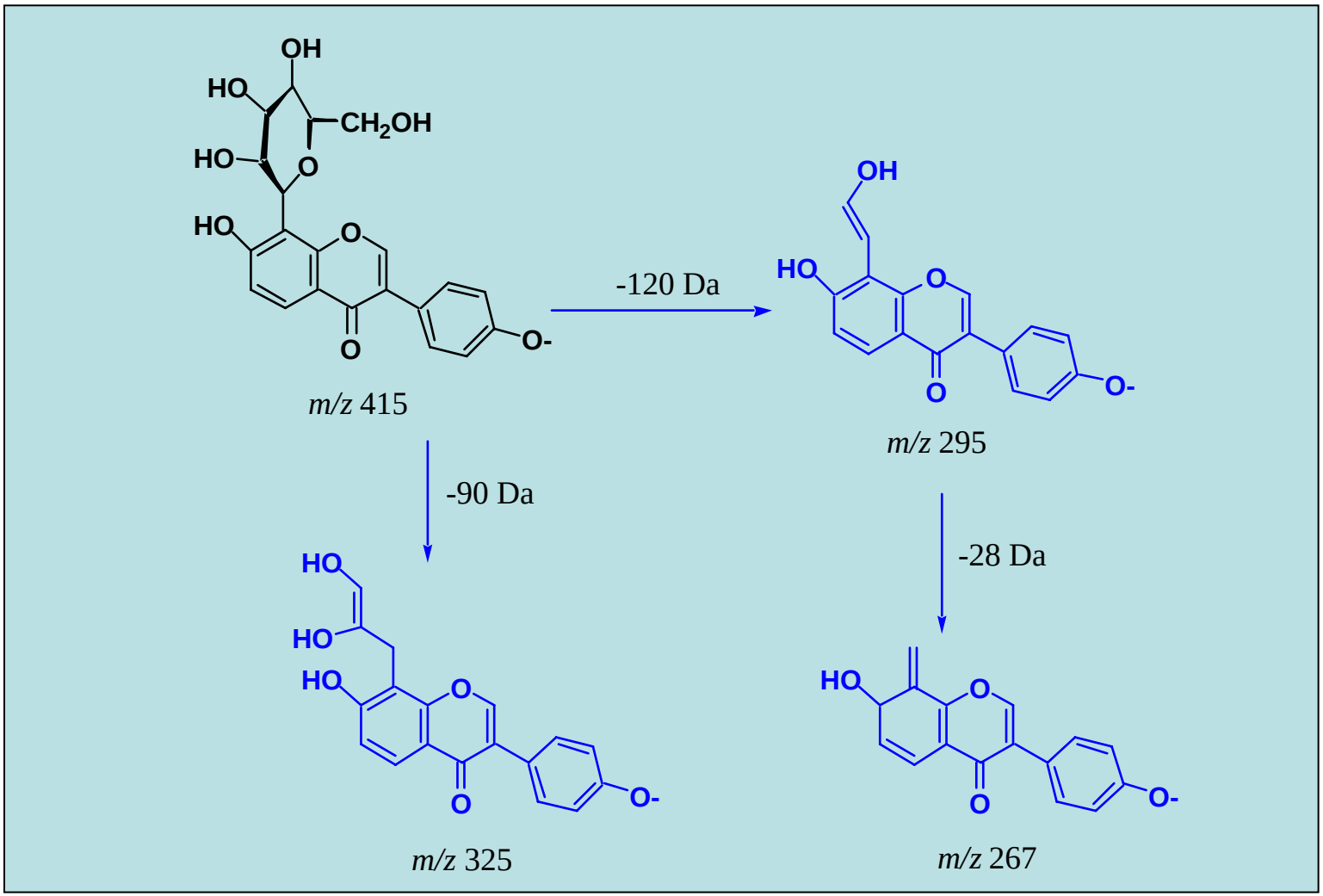


Neutral losses of 162 and 120 Da are characteristics

Proposed fragmentation mechanisms of protonated puerarin and daidzin

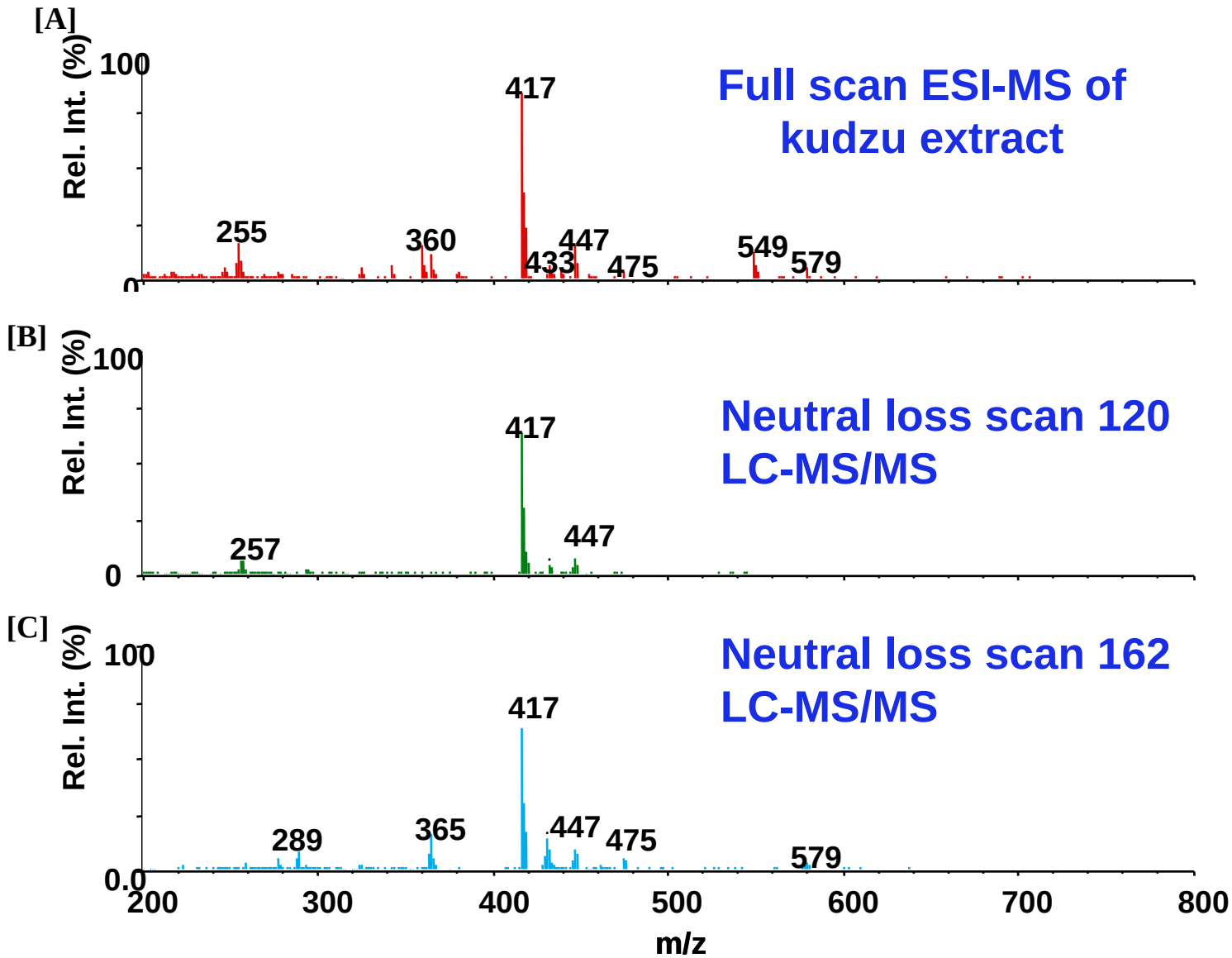


Possible product ions of puerarin in ESI-MS/MS in negative ion mode

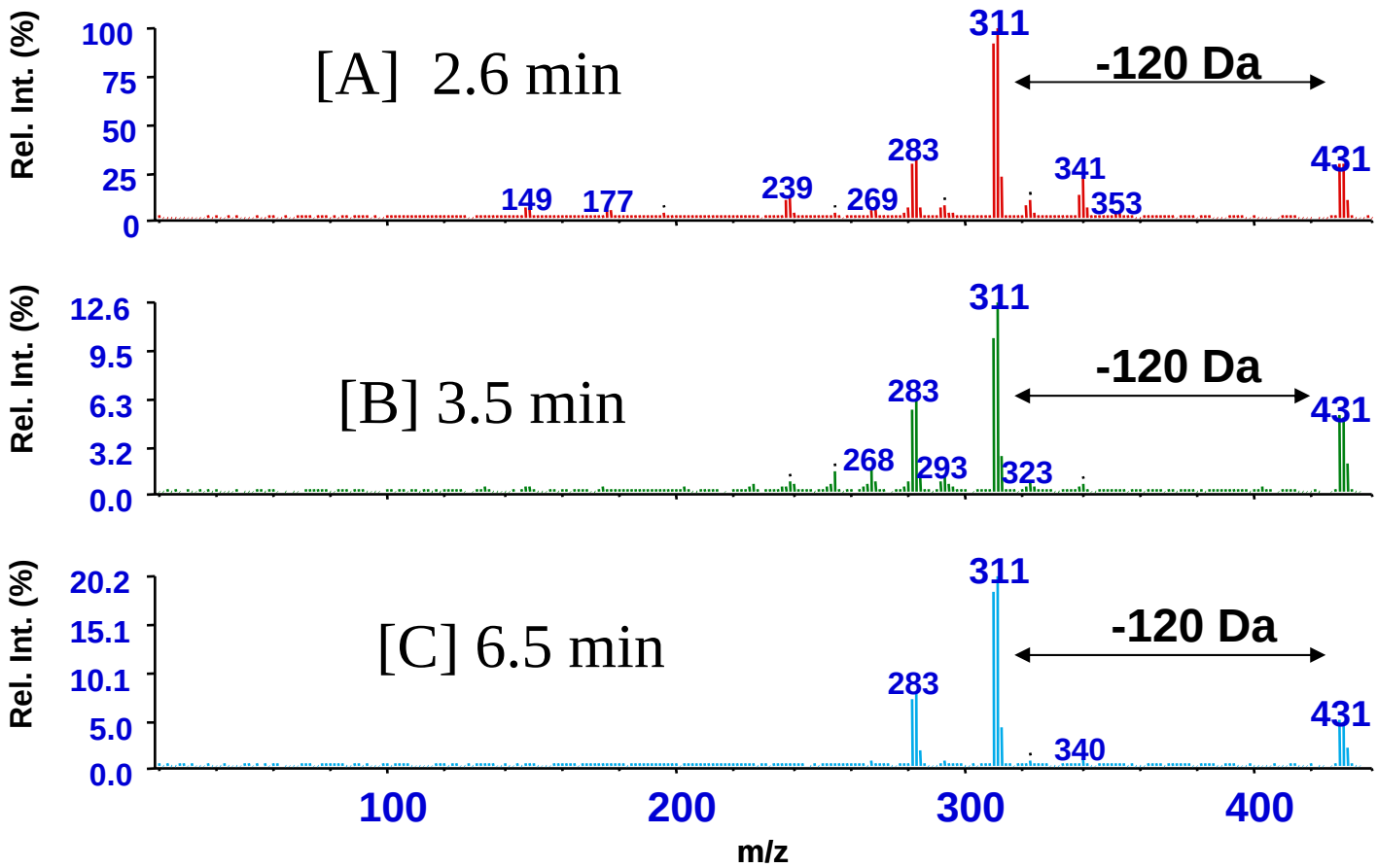


Prasain et al. J Agric Food Chem. 51, 4213, 2003.

Neutral loss scans 120 and 162 can be used to detect C-and O-glycosides, respectively in a crude mixture



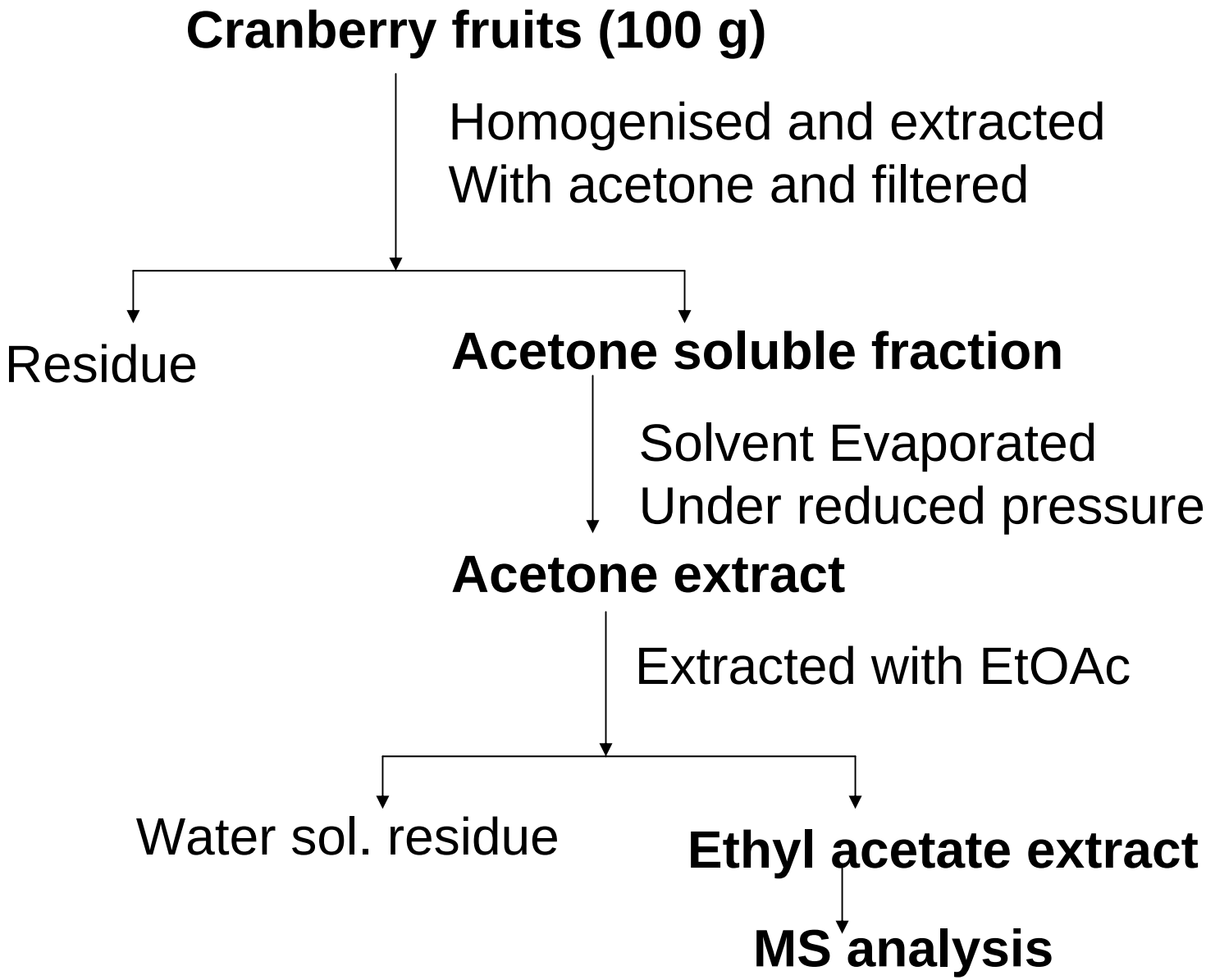
Hydroxylated daidzein C-glucosides were detected in the kudzu root culture extract



Prasain et al. Phytochemical Analysis, in press

LC-MS analysis of cranberry fruits/concentrate

Extraction Flow Chart of an ethyl acetate extract from cranberry fresh fruits



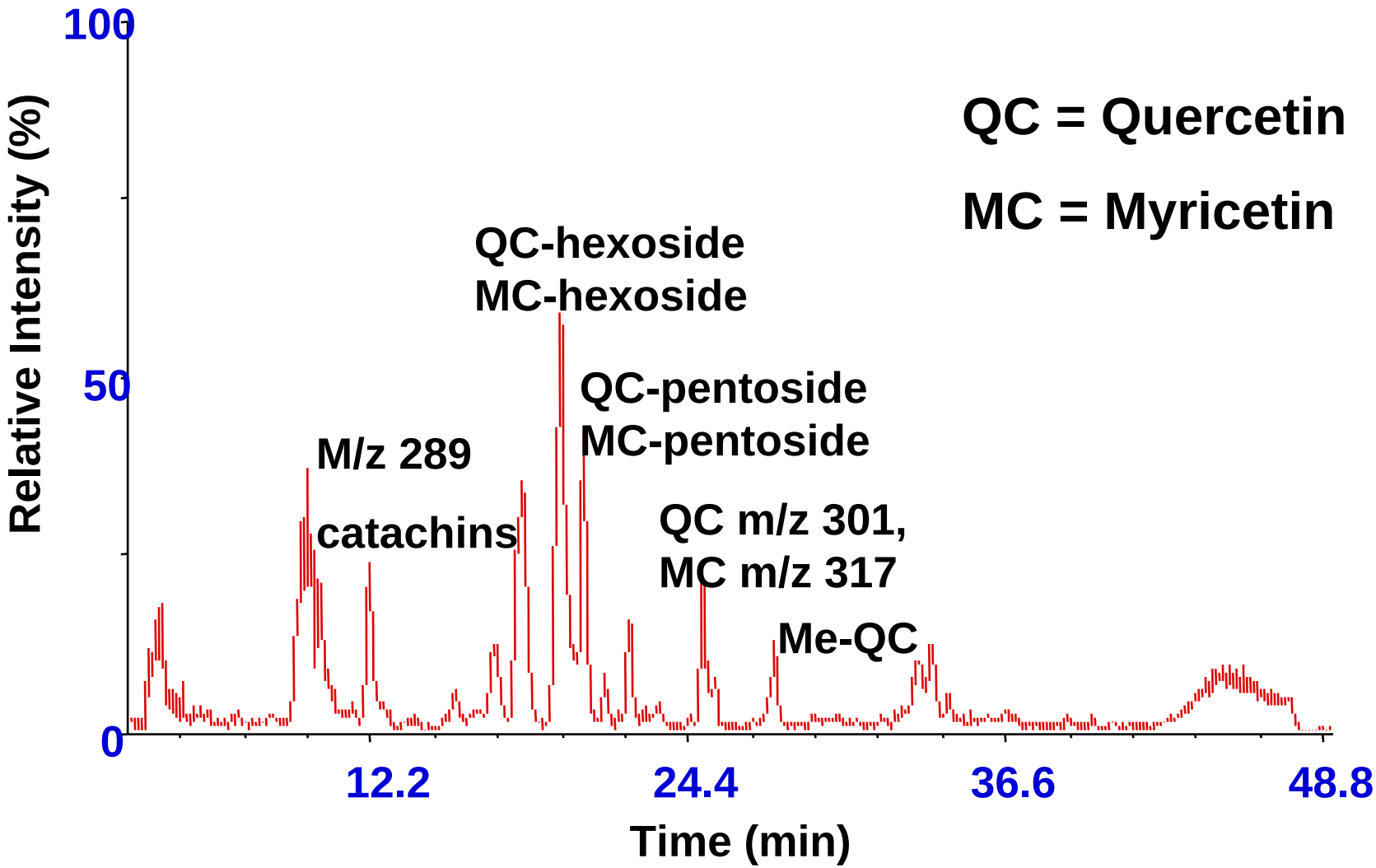
LC-MS and HPLC chromatographic conditions:

LC-MS column: 4.6 x 100 mm, RP-300 column
Gradient A : 10% ACN + 10 mM NH4OAc
B : 40% ACN + 10 mM NH4OAc

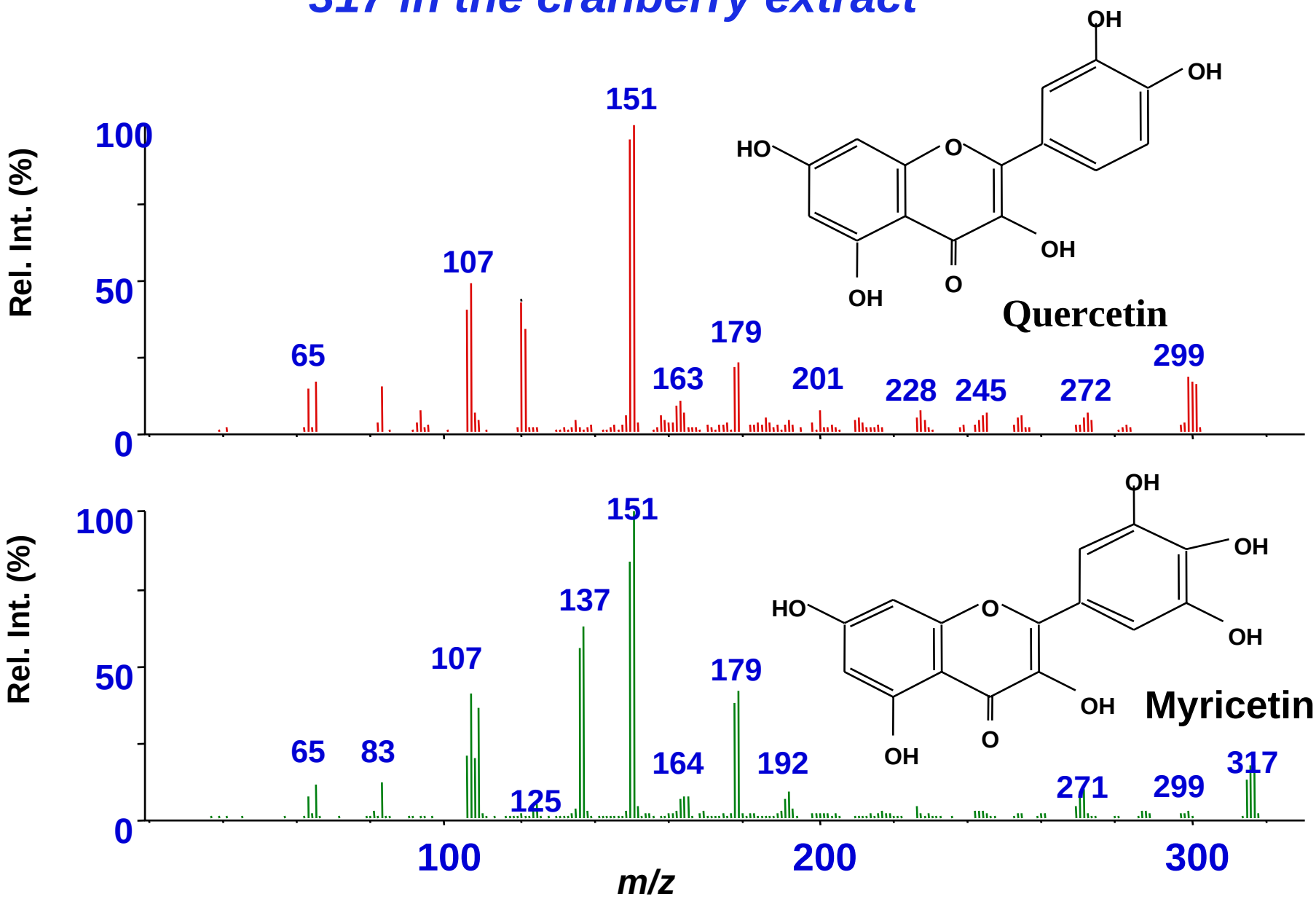
Linear gradient increased of solvent B 100% over 40 min
ESI-MS in the negative ion mode of a PE-Eciex API III
Triple quadrupole mass spectrometer.

HPLC analysis:
Column : Brownlee (22 cm x 4.6 mm I.d.) C8-column
Gradient A : 10% aq. ACN/0.1% TFA
B : 90% aq. ACN/0.1% TFA
Flow rate : 1.5 ml with a linear gradient increase of solvent B 100% over 35 min

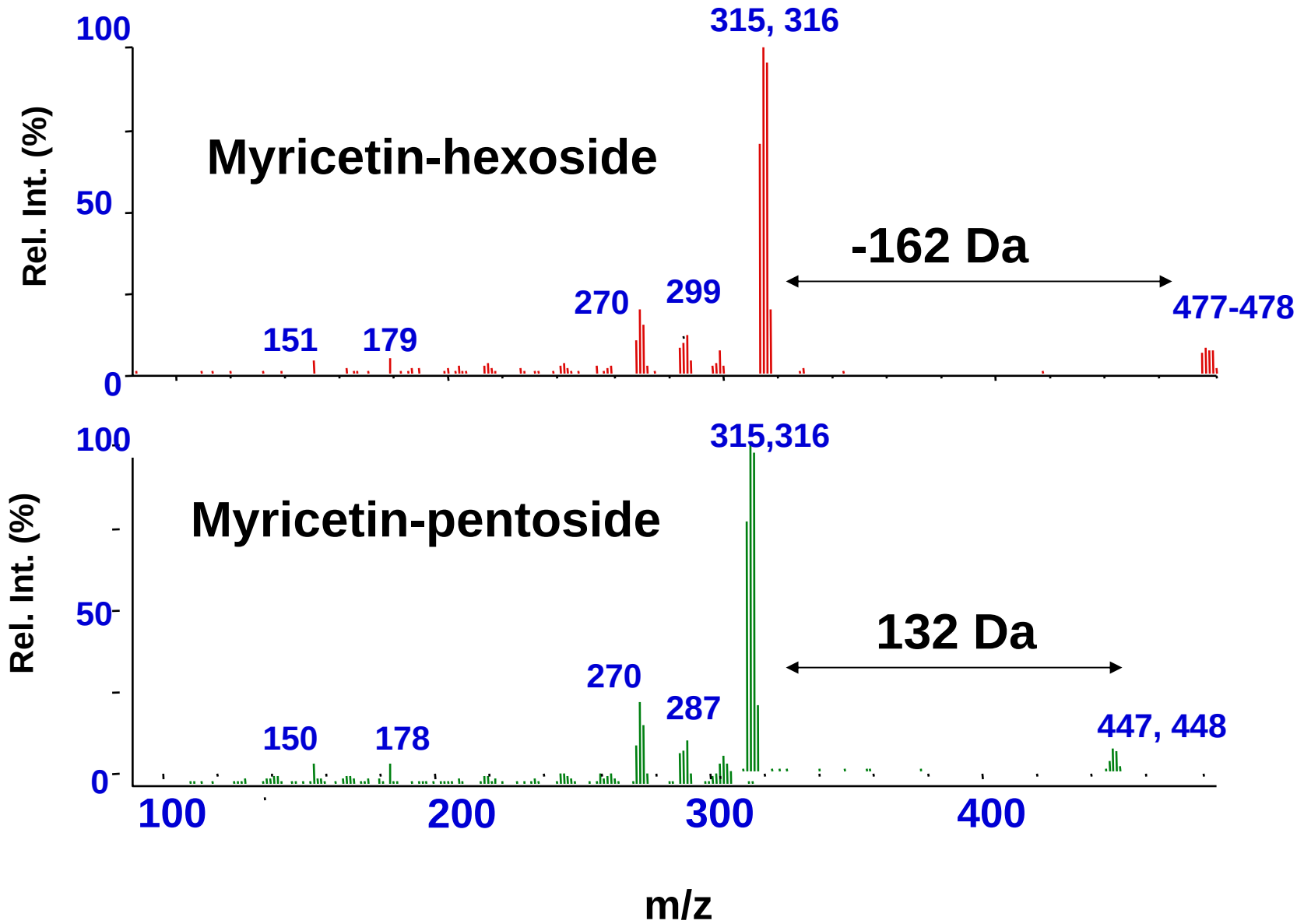
LC-MS chromatogram of the EtOAc extract obtained from Cranberry fresh fruits



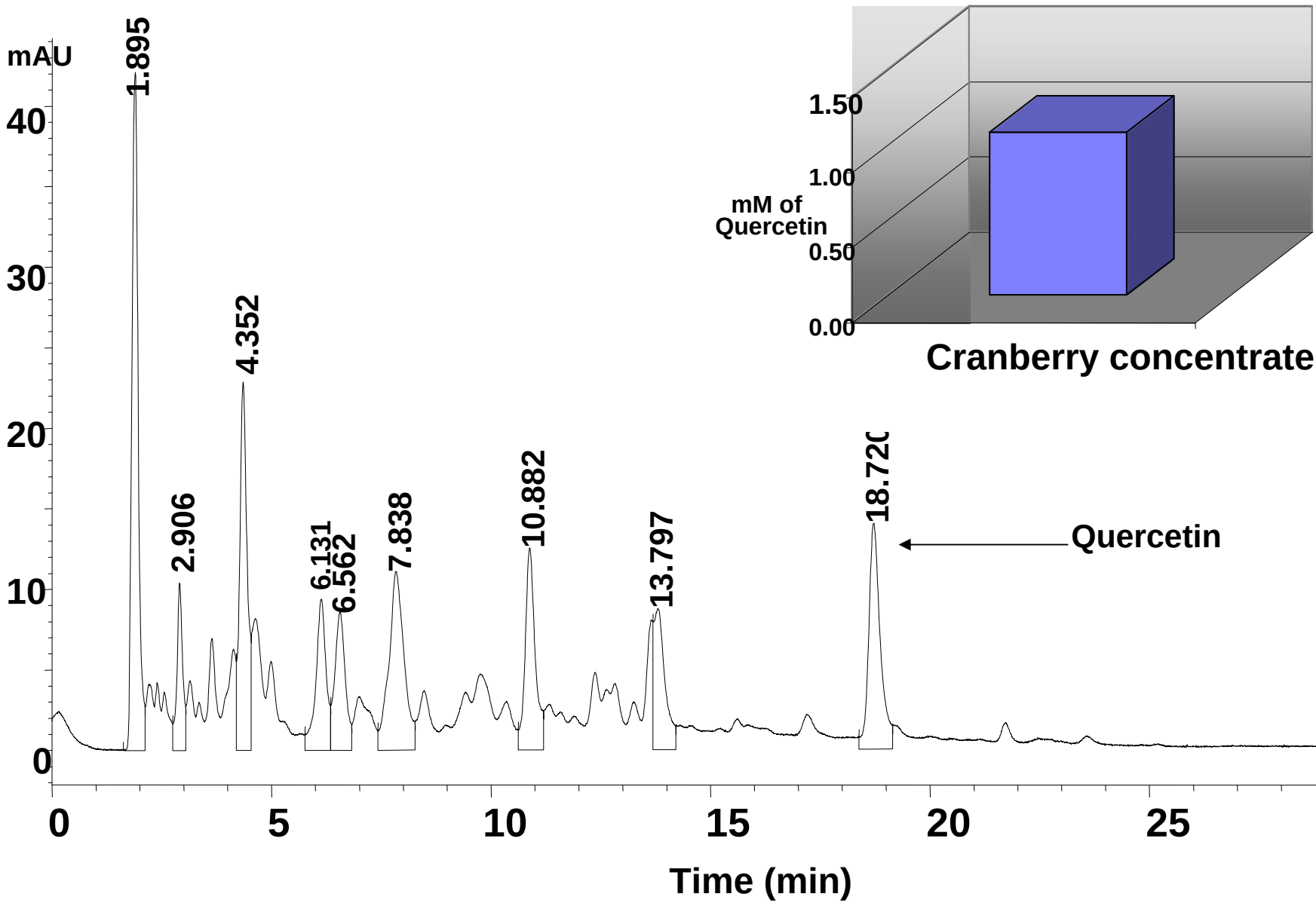
ESI-MS/MS spectra of the ions at m/z 301 and 317 in the cranberry extract



Pentose and hexose sugar derivative of Myricetin were detected the cranberry extract



HPLC Chromatogram of a commercially available cranberry concentrate

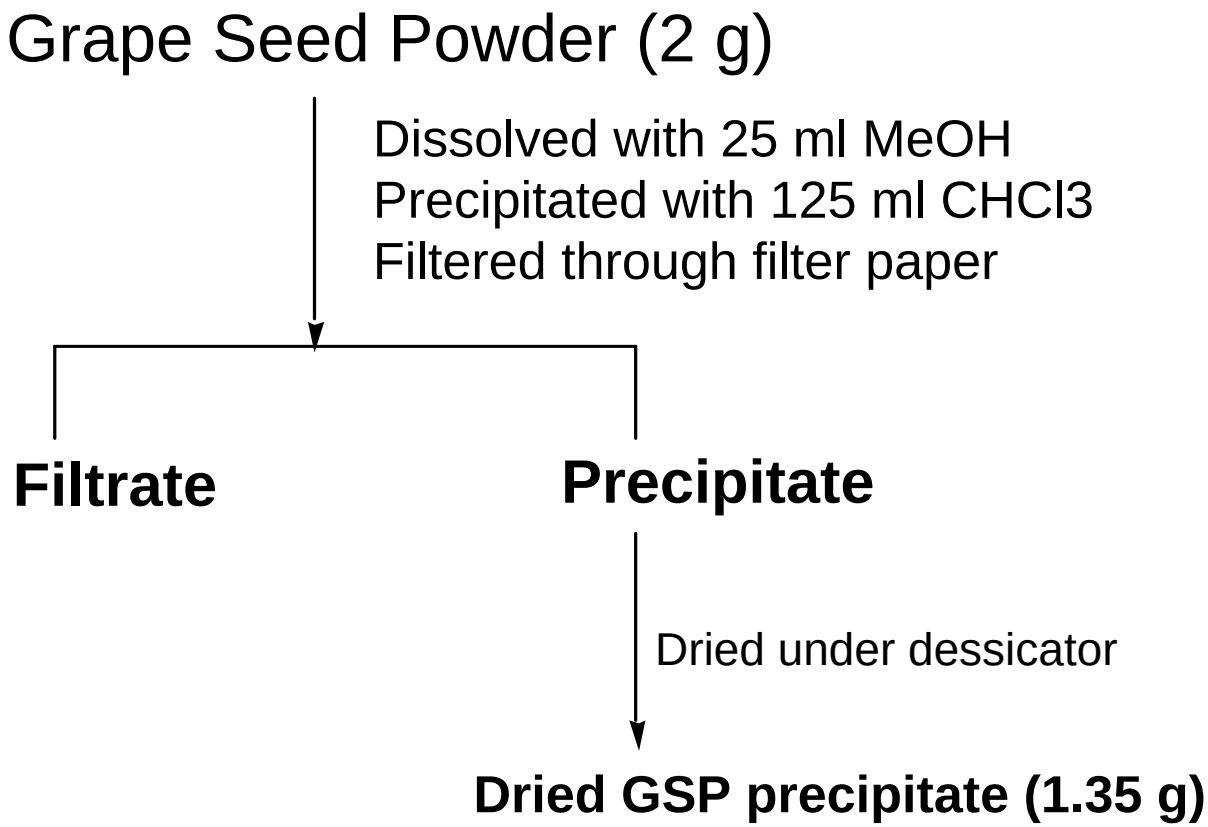


Conclusions

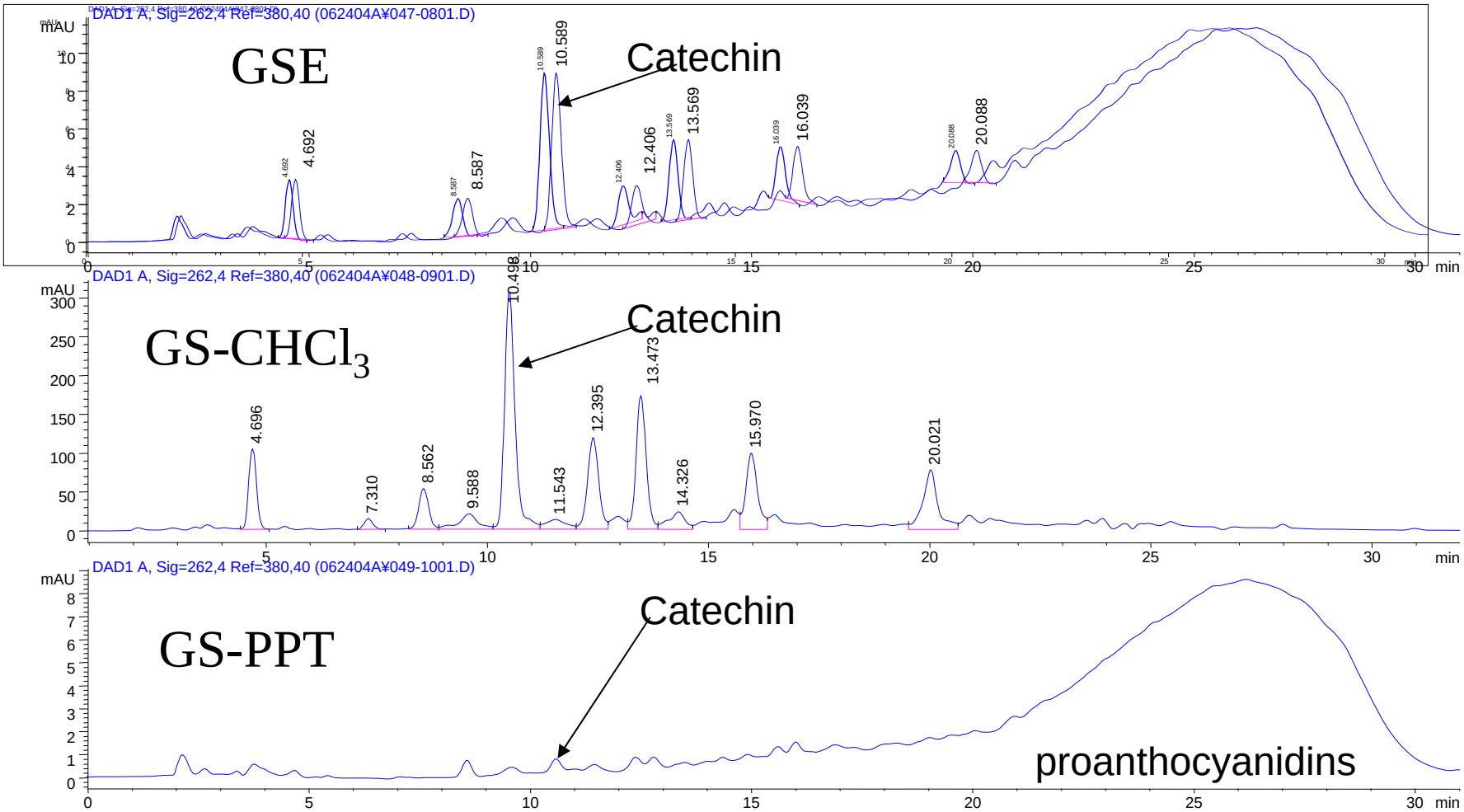
- **Quercetin and its glycosides are the major flavonoids in the cranberry fresh fruit, and commercially available cranberry concentrates.**
- **Catechin was detected but its oligomers (proanthocyanidins) were not detected in the ethyl acetate extract of the samples.**
- **Quercetin, myricetin and their glycosides produced predominant radical anions in the ms/ms experiments.**

Analysis of Grape Seed Extracts (GSE)

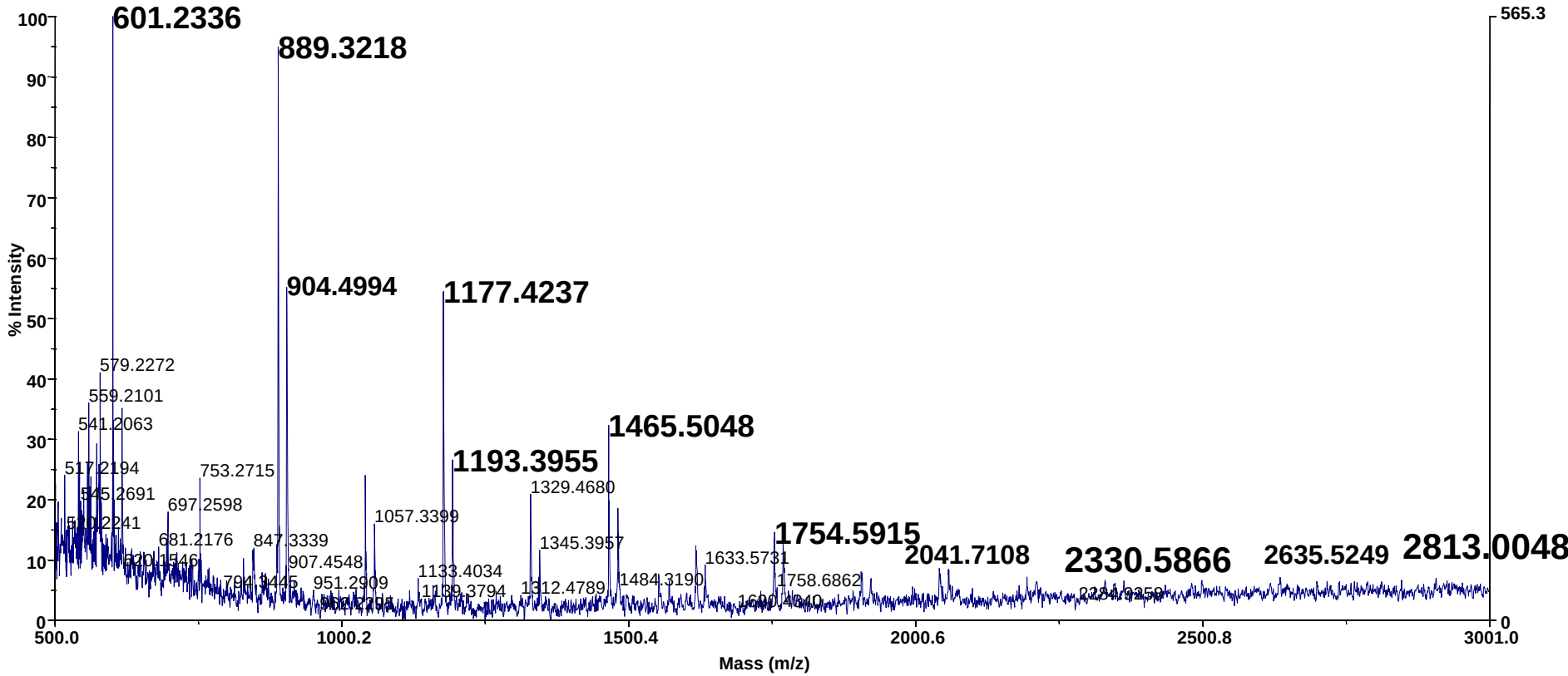
Fractionation of monomeric catechins from oligomers



GSE is a complicated mixture of polyphenols And other secondary metabolites



MALDI-TOF mass spectrum in positive linear mode showing a pronathocyanidin series [M+Na]+ from the dimer m/z 600 to oligomers



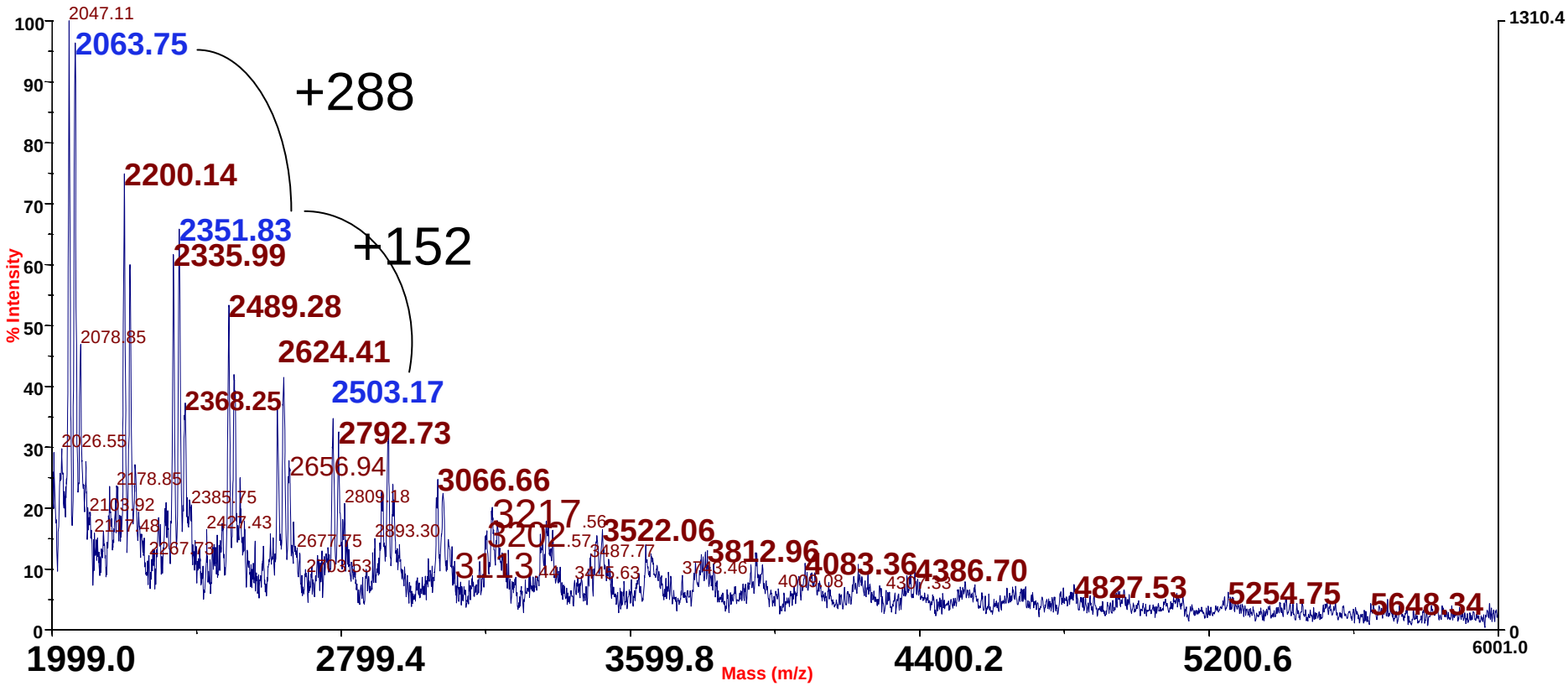
Equation to detect poly-galloyl-polyflavans in GSP

290 + 288c + 152g + 23

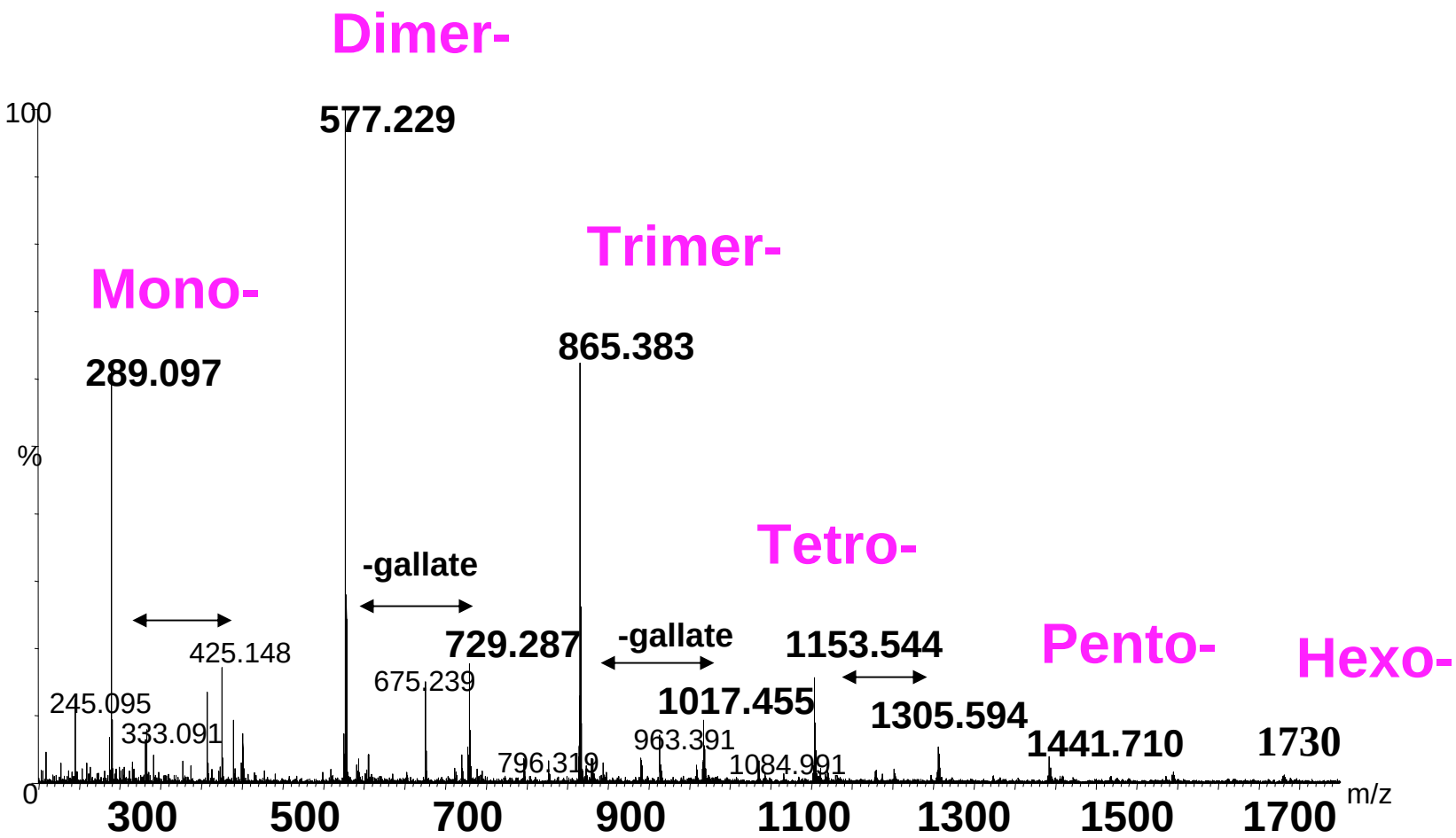
290 = mol wt. of the terminal catechin/epicatechin

C = degree of plymerization

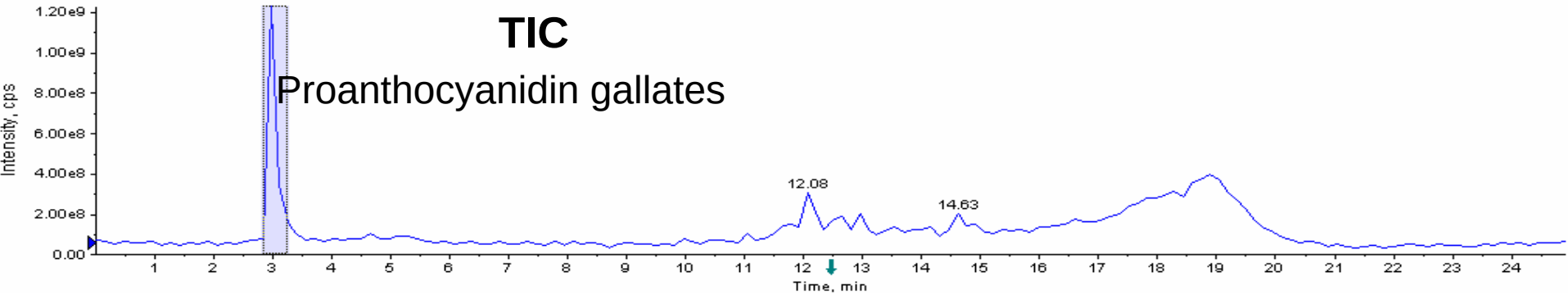
G = unit of galloyl esters; 23 = weight of sodium

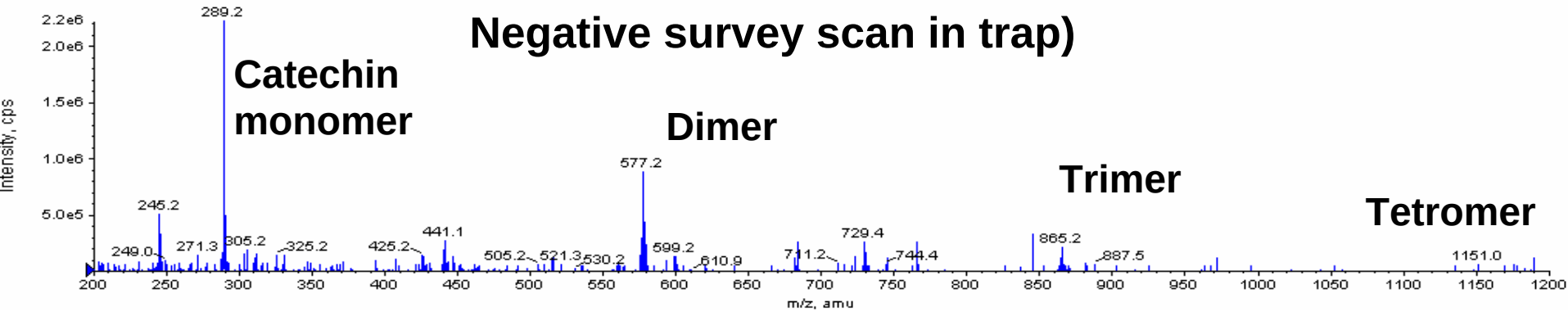


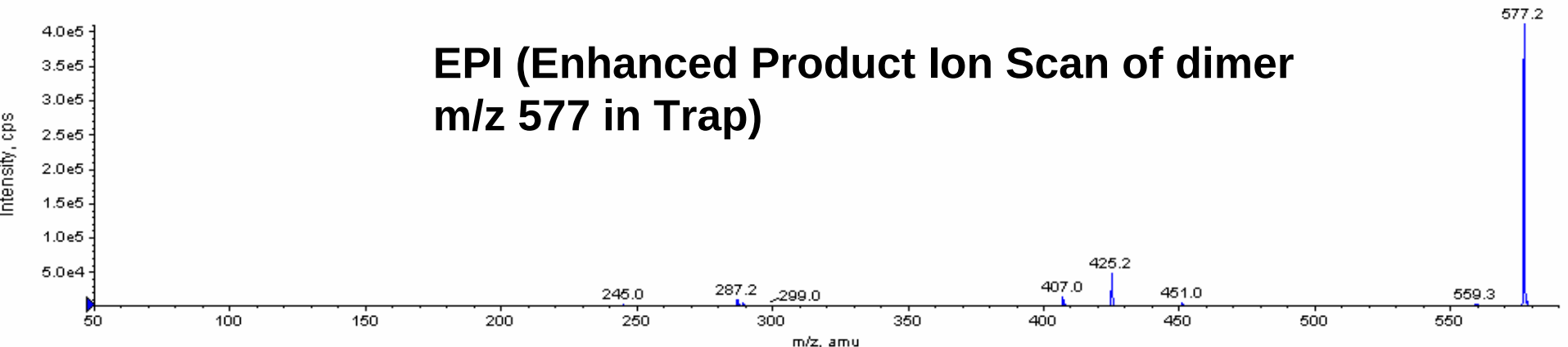
GSE precipitate in ESI-MS QTOF



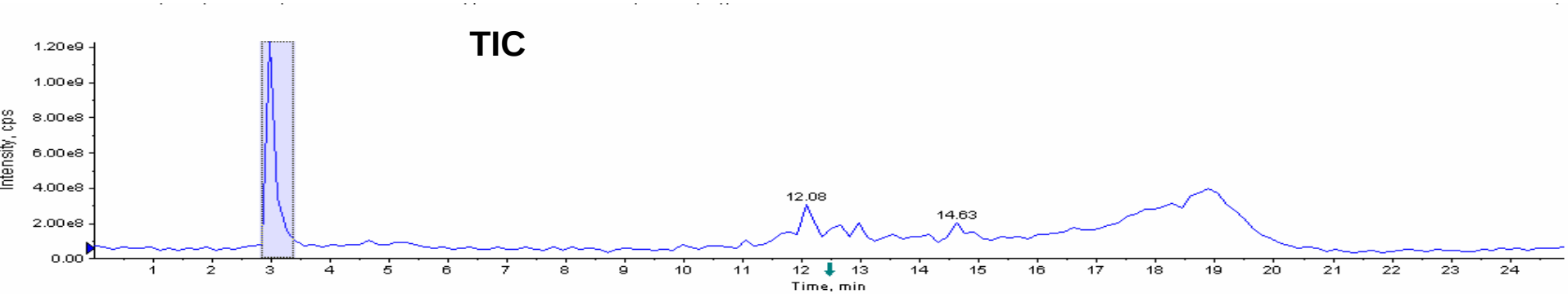
LCMS and MSMS analysis of the methanolic extract of a Grape Seed Dietary Supplement with ionization polarity

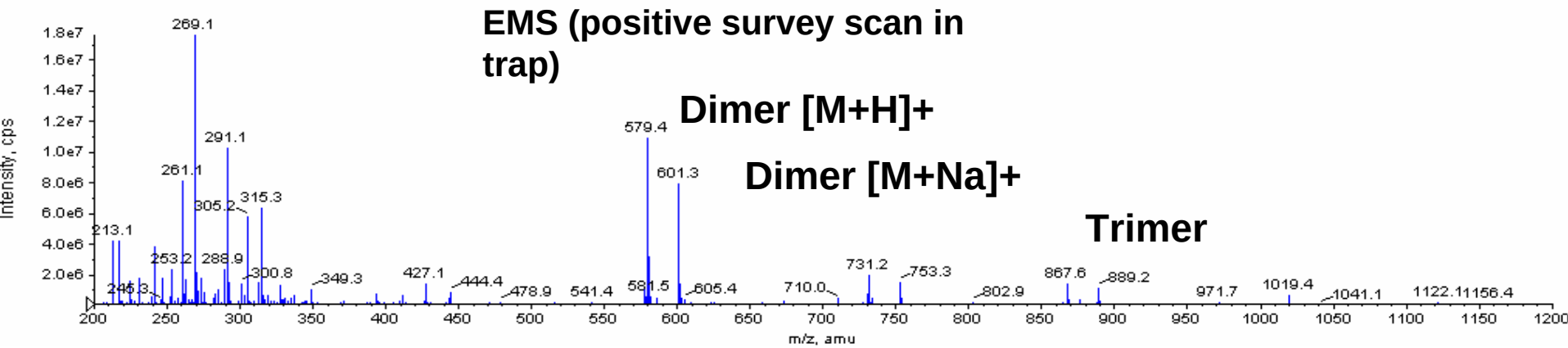


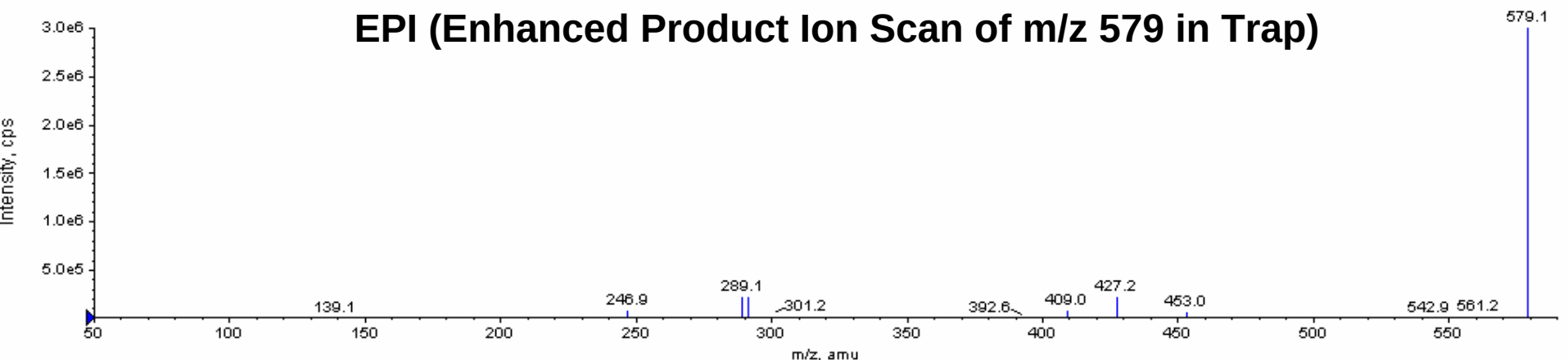




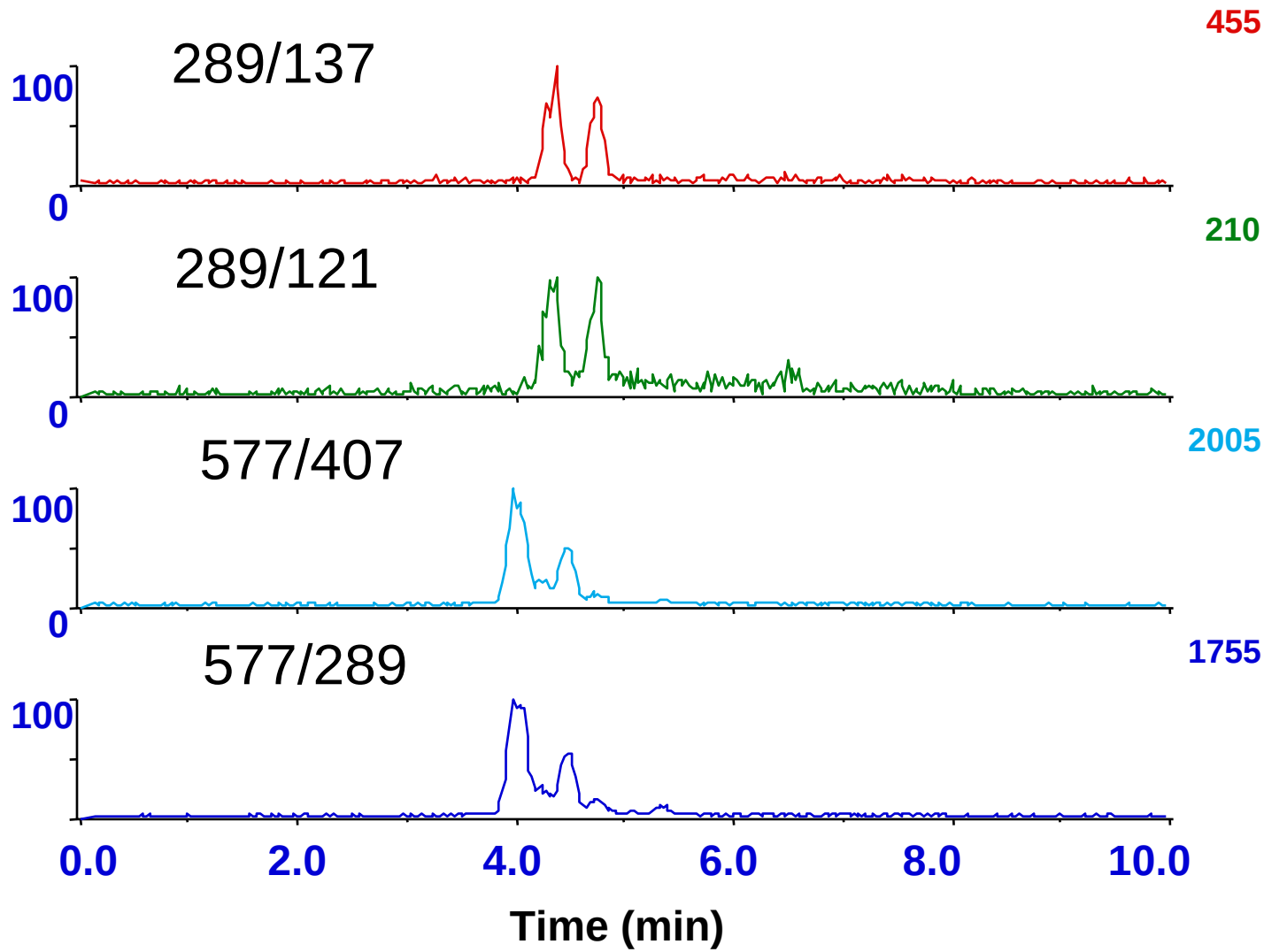
Metal ion adducts are common in Positive Mode





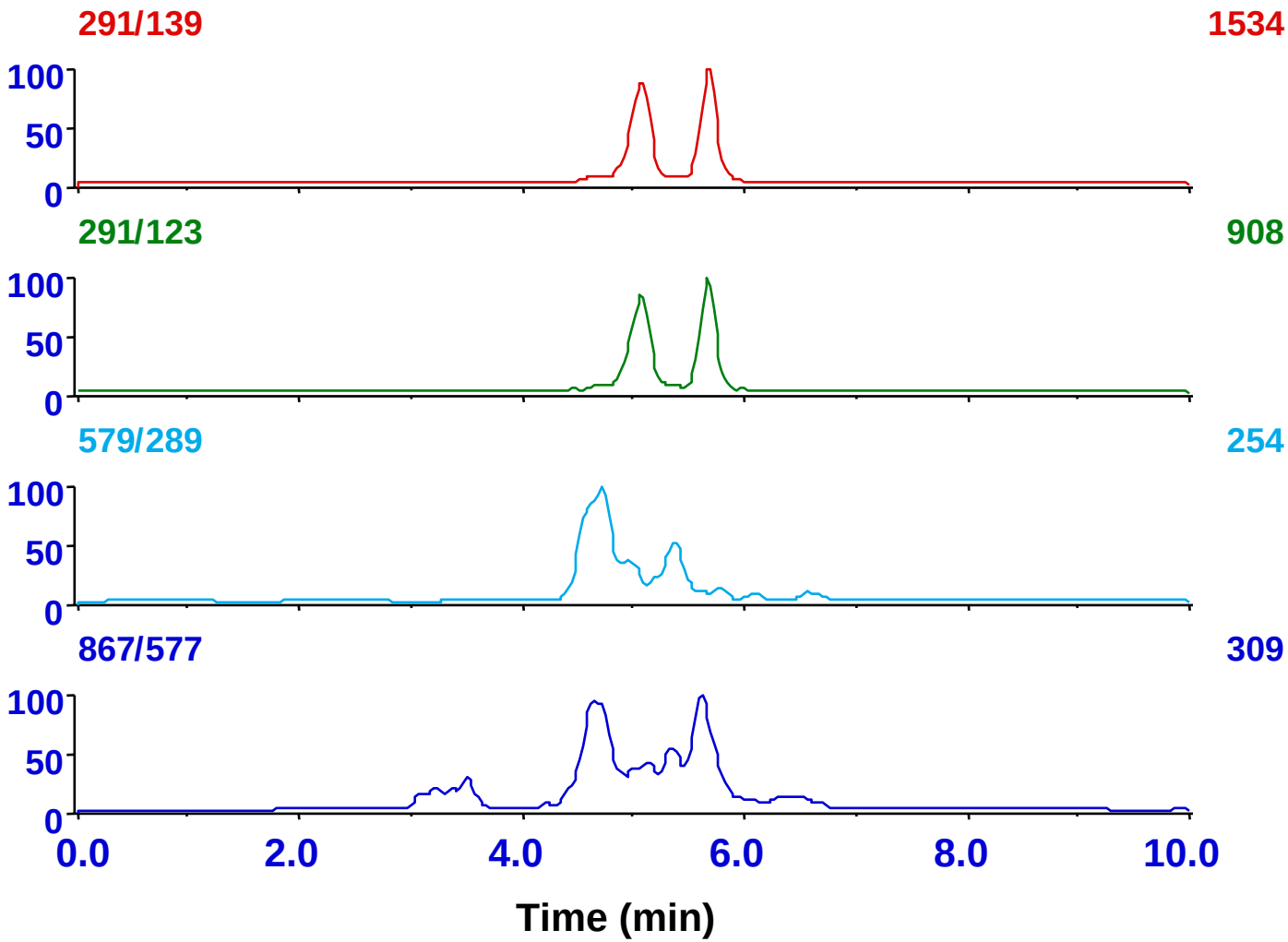


Negative ion mode provides better sensitivity for dimer than monomeric catechin



Gradient: 10 min from 0-60% MeOH in 5 min in 0.1% HCOOH

Monomeric catechin is better detected in positive ion mode



Conclusions

A sensitive LC-MS/MS method was developed to analyze GSP in biological samples. In mass spectrometric analysis, monomeric catechins are more sensitive in positive ion mode, while catechins dimers are in negative ion mode.

GSP is a complex mixture of catechins and non-polyphenols.

Thanks to…

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