

Phytochemicals: Identification by Mass Spectrometry and NMR

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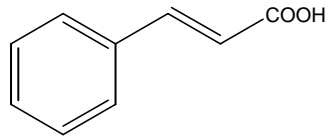
Why Structural Elucidation?

- Current EU regulations on nutrition & health (2006) requires:
 - isolation & purification
 - chemical structure
 - chemical synthesis
 - dose-response activity curves

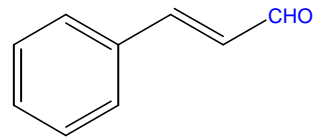
Analytical challenges in phytochemistry

- Chemical diversity
- Dynamic range
- Distribution (Spatial/temporal)
- Little help from gene bank

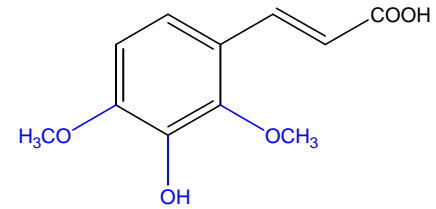
Diversity of phytochemicals



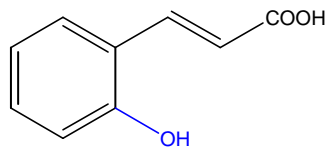
$C_9H_8O_2$
Mol. Wt.: 148.16



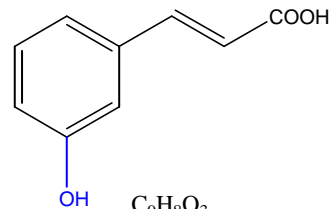
C_9H_8O
Mol. Wt.: 132.16



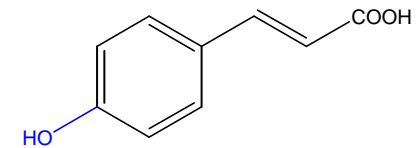
$C_{11}H_{12}O_5$
Mol. Wt.: 224.21



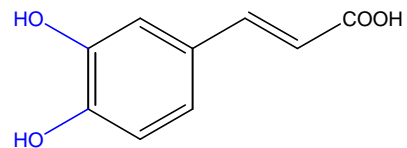
$C_9H_8O_3$
Mol. Wt.: 164.16



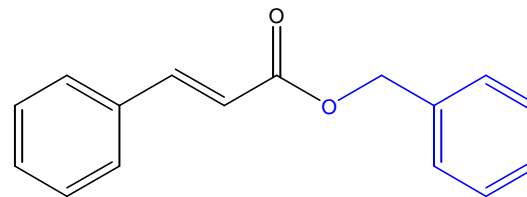
$C_9H_8O_3$
Mol. Wt.: 164.16



$C_9H_8O_3$
Mol. Wt.: 164.16

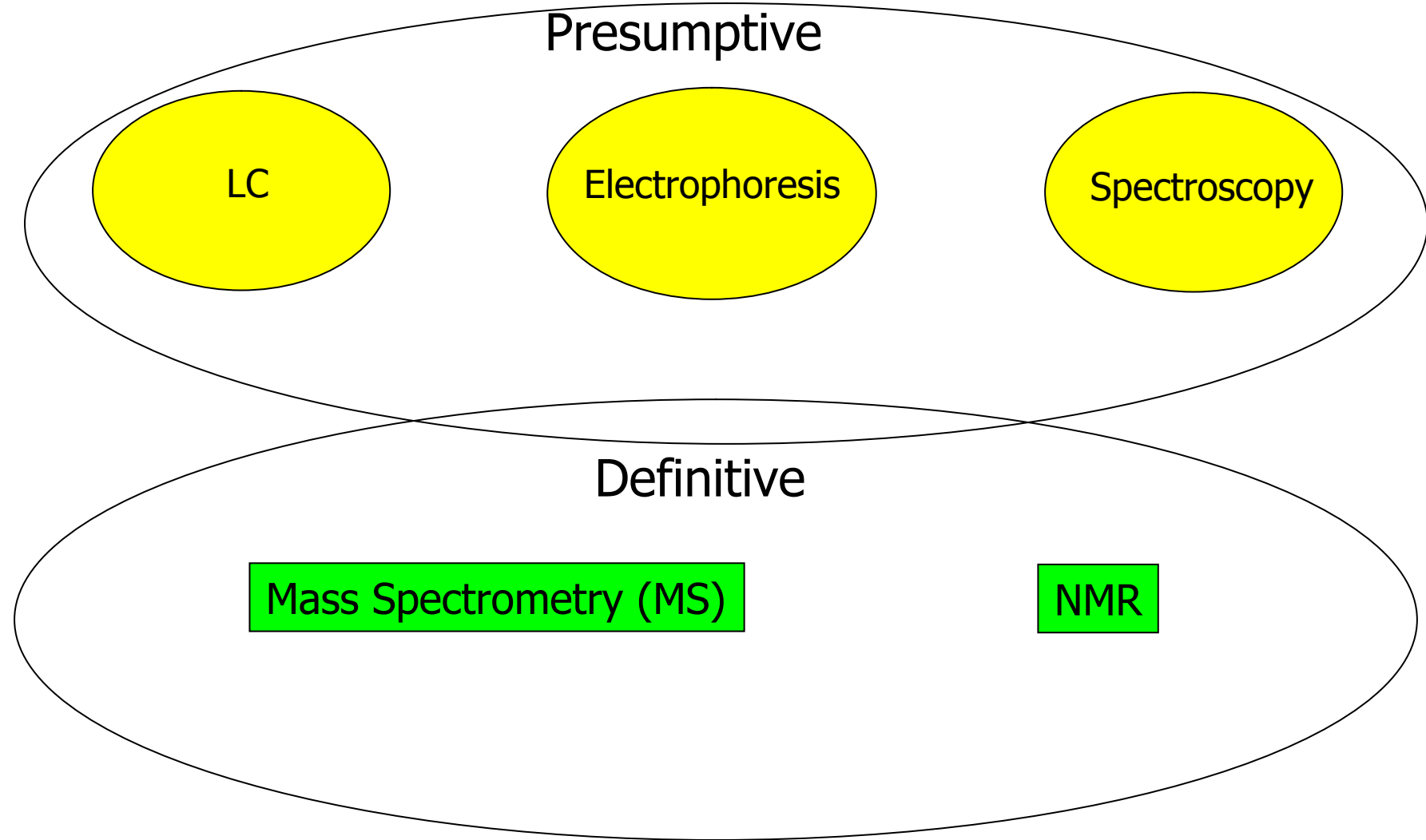


$C_9H_8O_4$
Mol. Wt.: 180.16



$C_{16}H_{14}O_2$
Mol. Wt.: 238.28

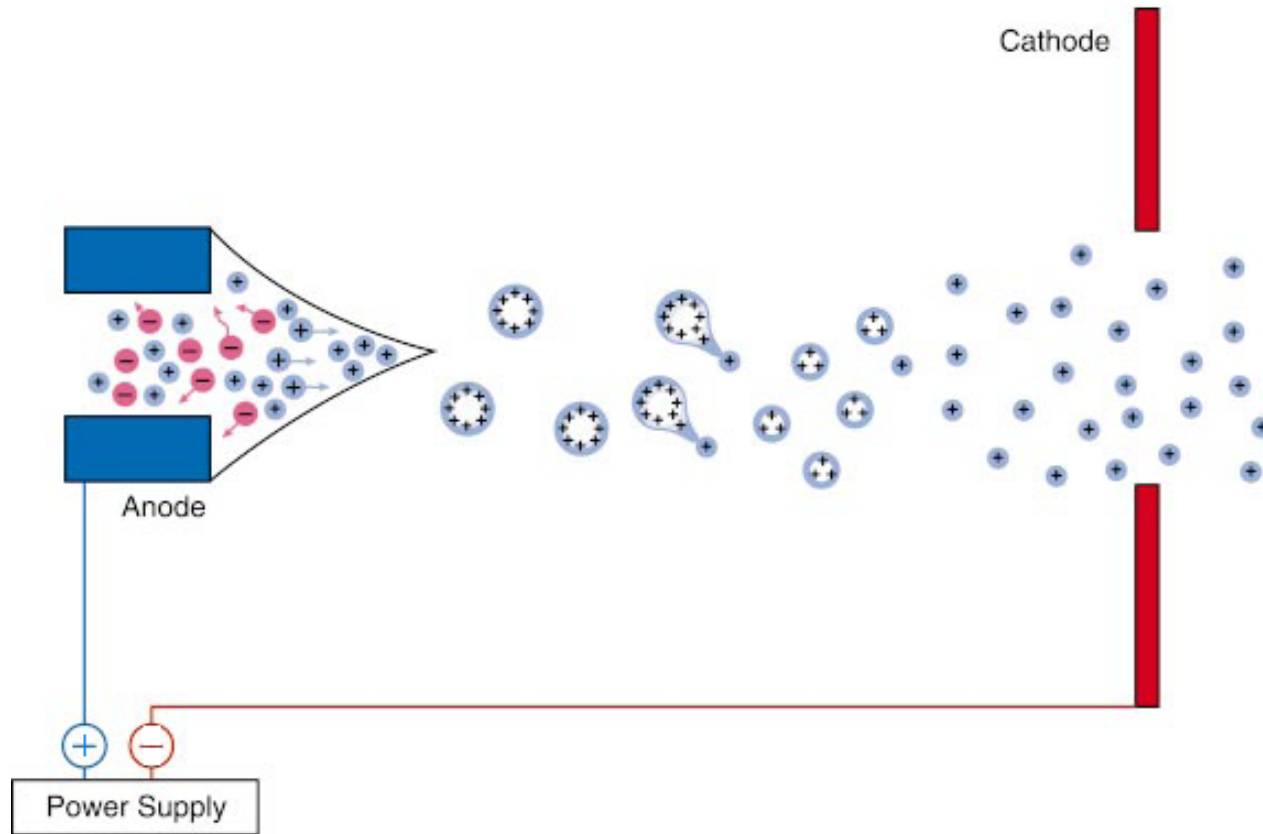
Analytical Techniques



Mass Spectrometry

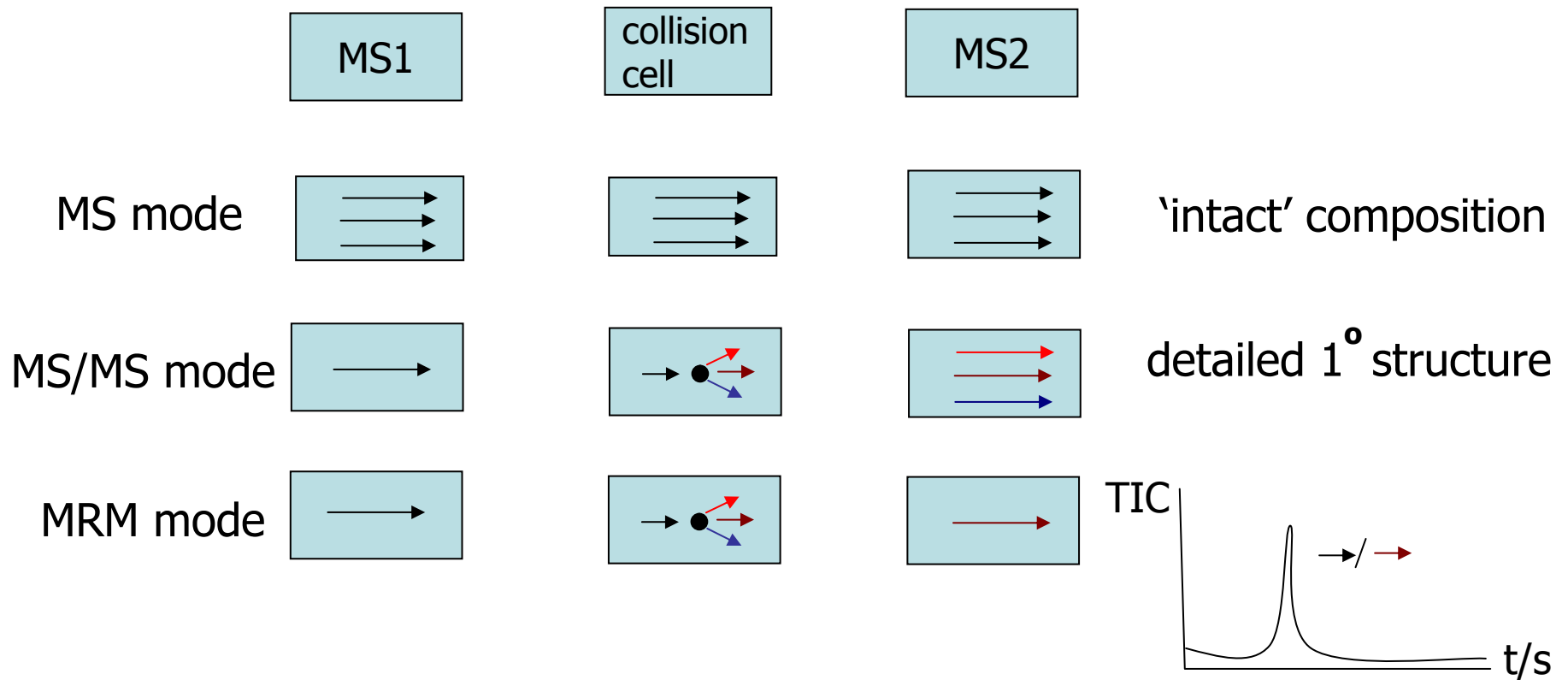
- measures mass-to-charge ratios (m/z)
- ionised molecules
- gas phase
- information about mass **independent of the shape** of the molecule

Electrospray Ionisation Process



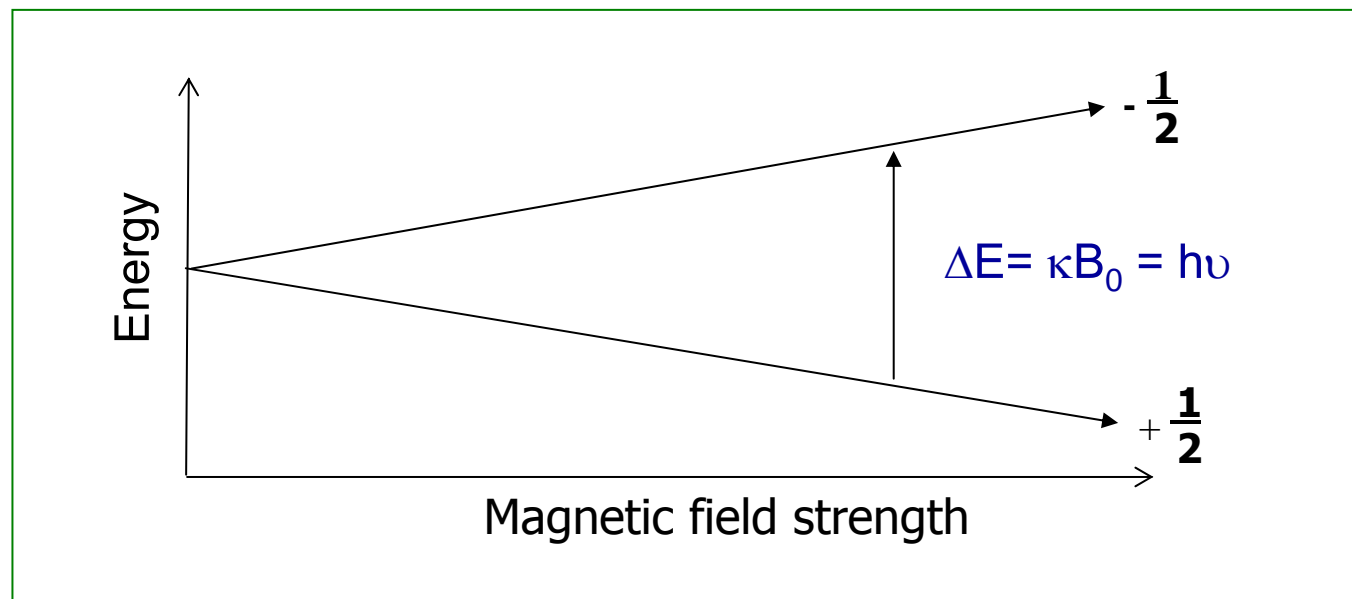
Griffiths WJ, Jonsson AP, Liu S, Rai DK, Wang Y. *Biochem J.* 2001; 355: 545-561.

Major Mass Spectrometric Experiments



NMR

- non-destructive method
- magnetic nuclei when pulsed with electromagnetic field; absorbs & radiates energy at a specific radio frequency



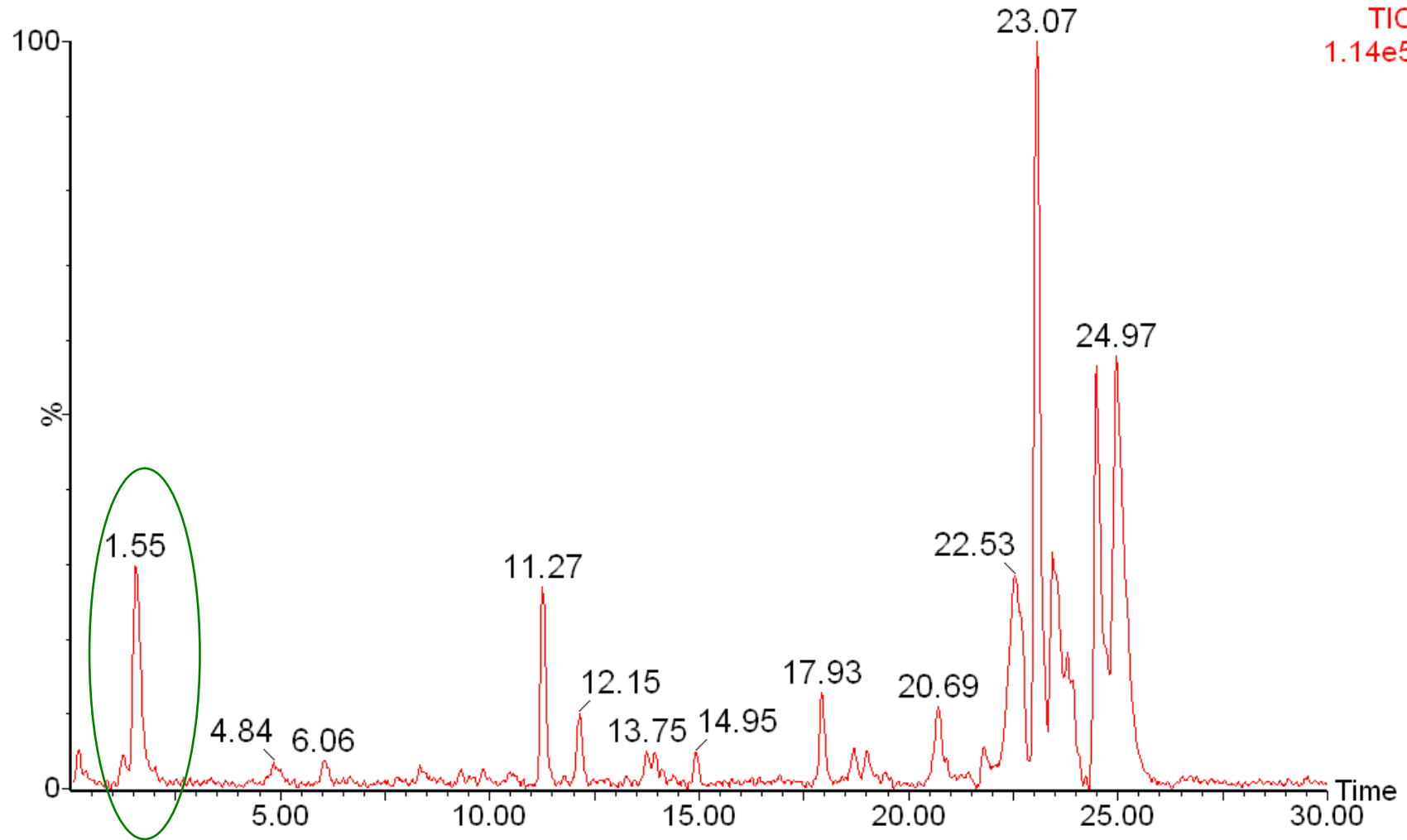
Major NMR experiments

- Proton (^1H) NMR
- ^{13}C -NMR
- 2D NMR, e.g. COSY ($^1\text{H} - ^1\text{H}$)

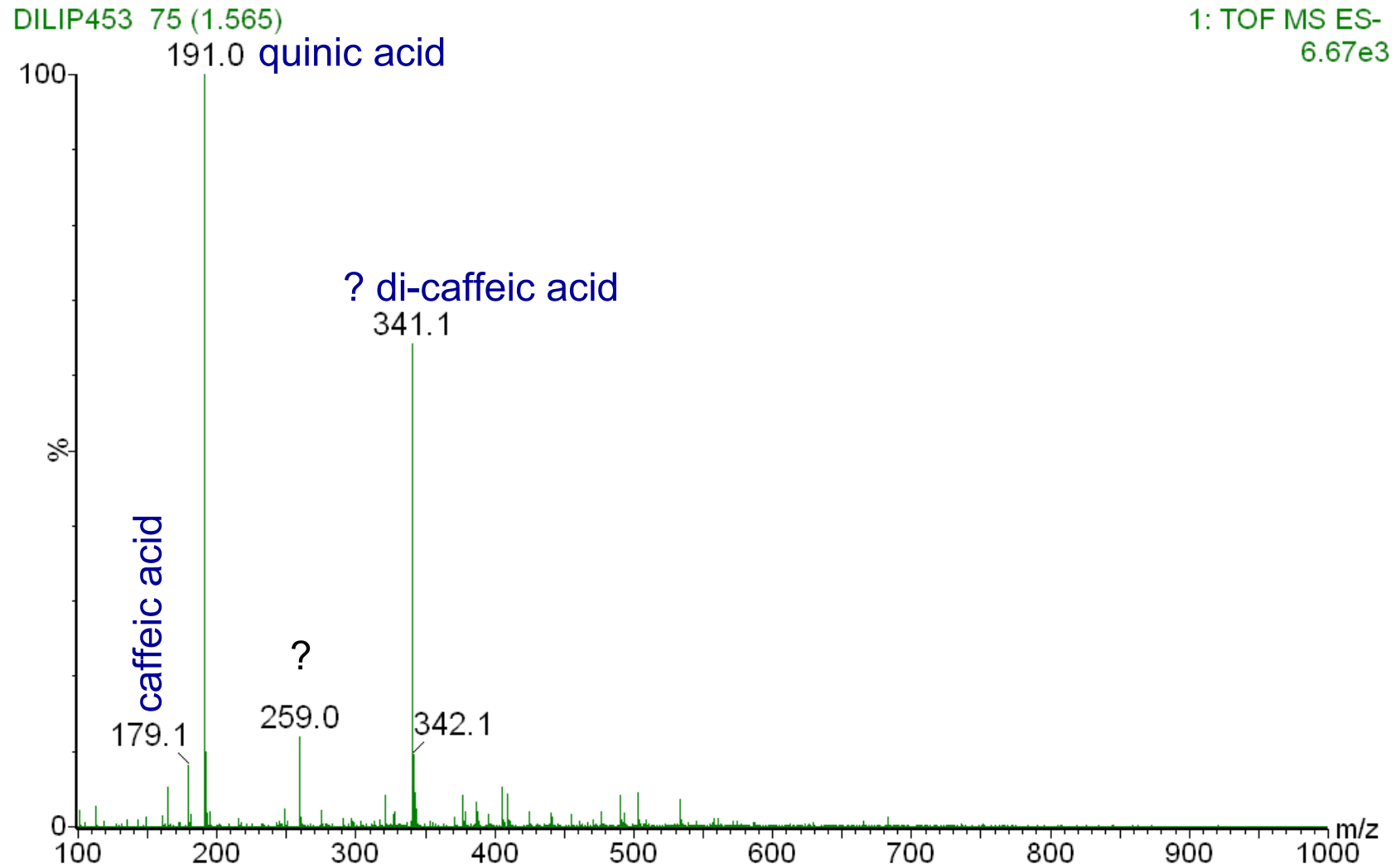
LC-MS of Rosemary Extract

Mohammad, R
DILIP453

1: TOF MS ES-
TIC
1.14e5



Mass Spectrum for LC peak eluting at 1.55 min.



Accurate Mass: Caffeic acid

Elemental Composition Report

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Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0

Selected filters: None

Monoisotopic Mass, Even Electron Ions

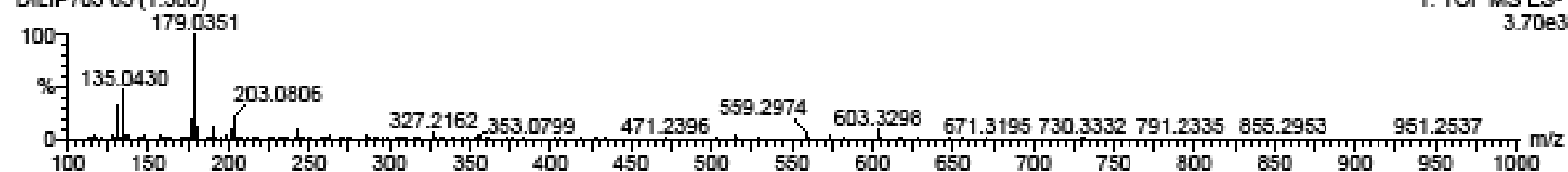
27 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 0-100 H: 0-100 O: 0-20

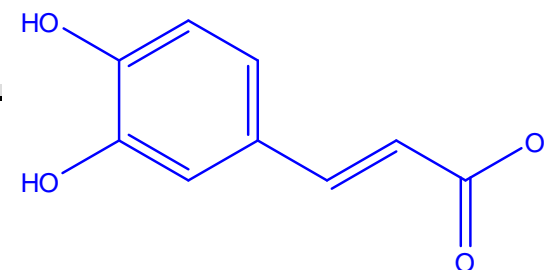
DILIP703 65 (1.368)

1: TOF MS ES-
3.70e3



Minimum: -1.5
Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
179.0351	179.0344	0.7	3.9	6.5	15.9	C9 H7 O4

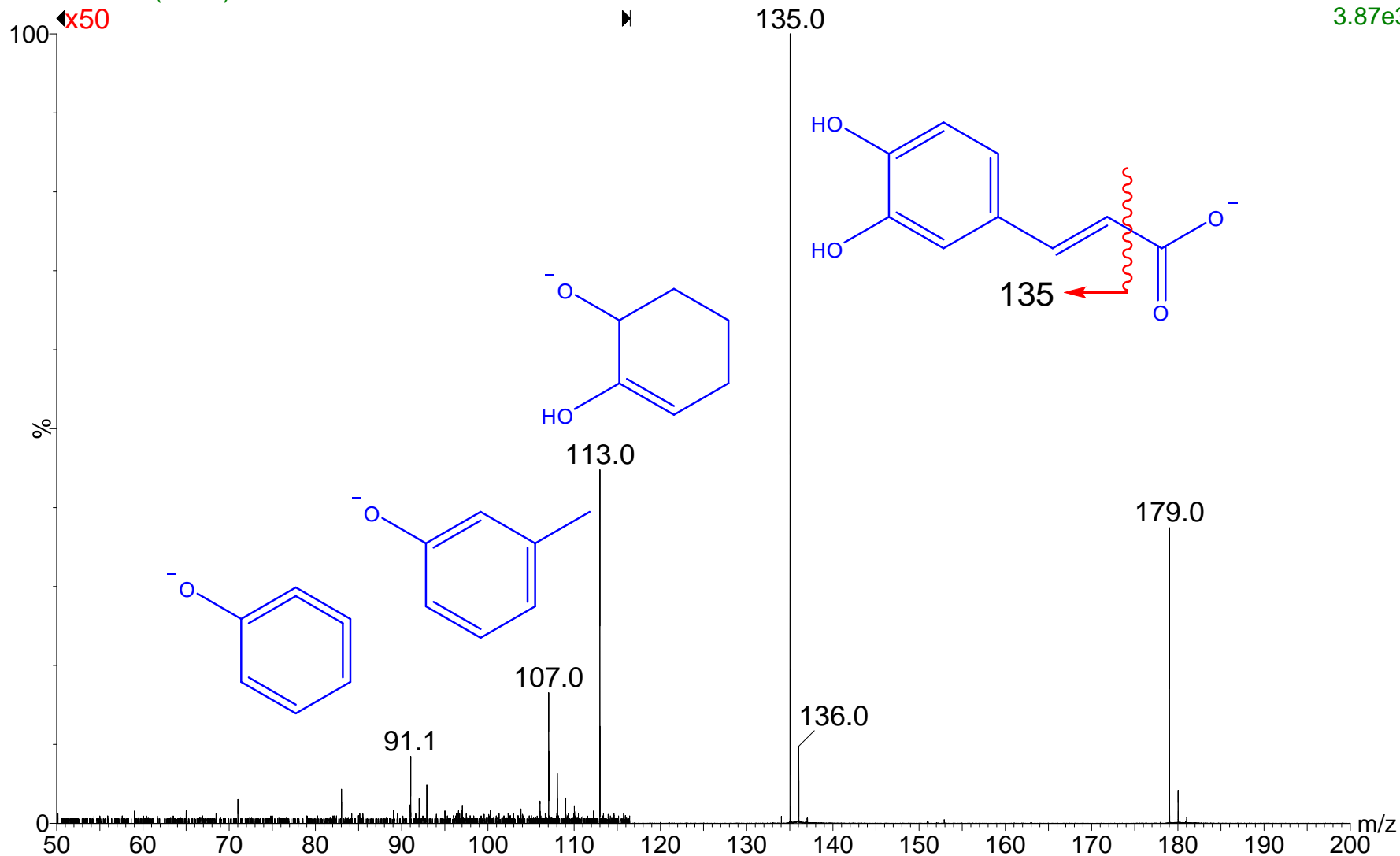


Chemical Formula: C₉H₇O₄⁻

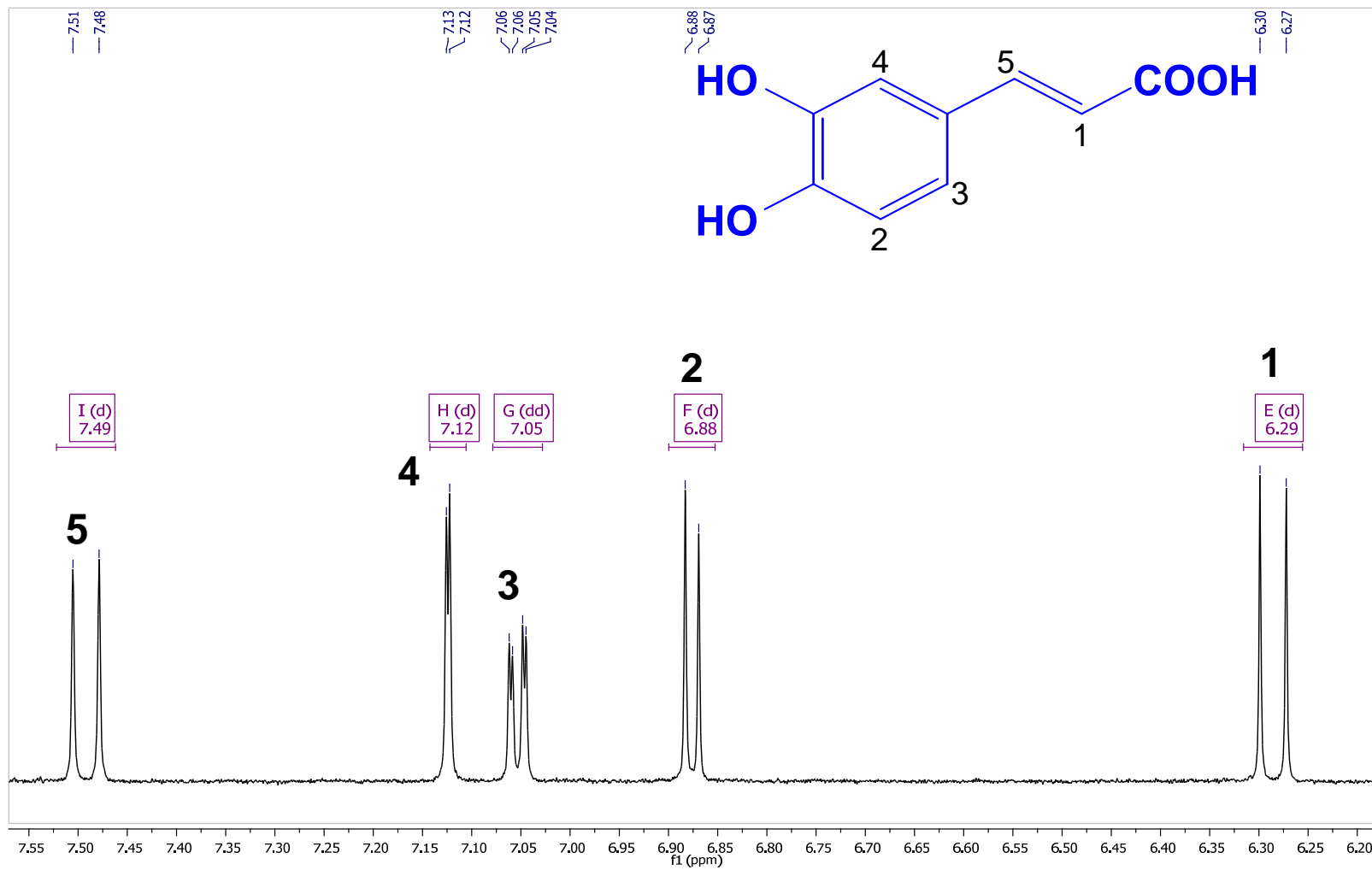
MS/MS of Caffeic acid

DILIP668 18 (1.712)

2: TOF MSMS 179.04ES-
3.87e3

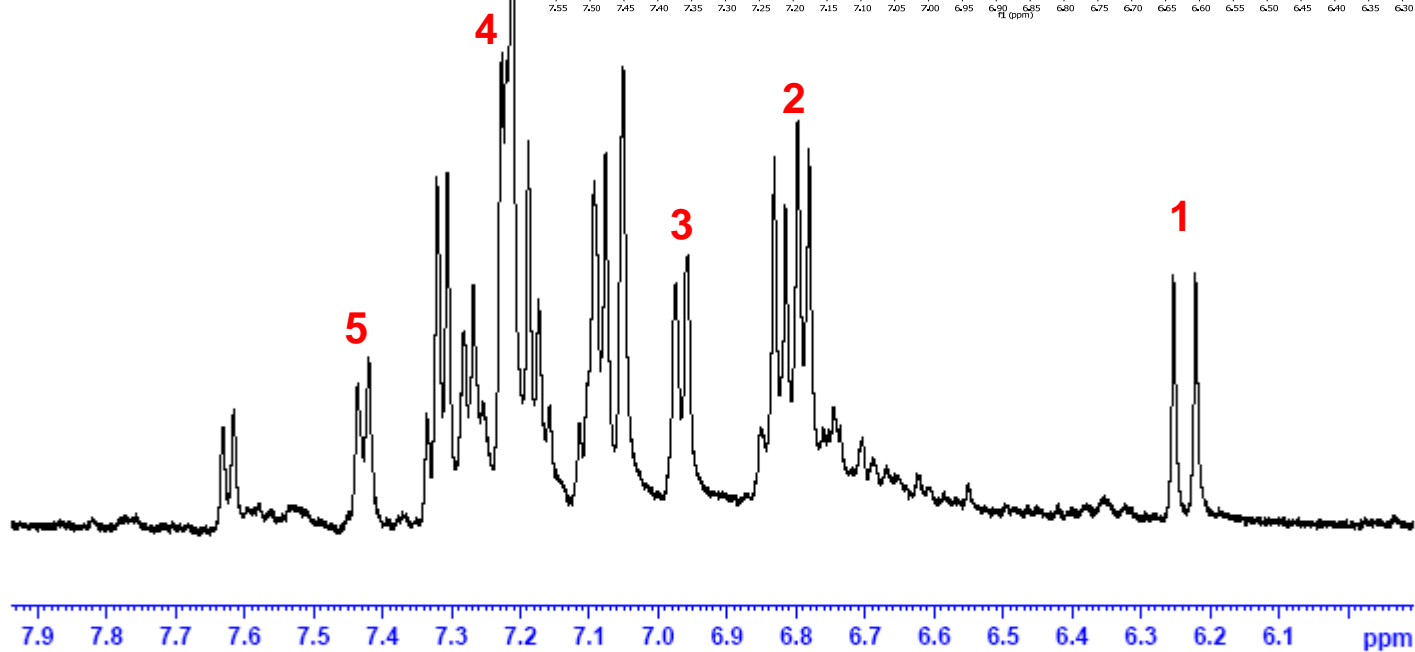
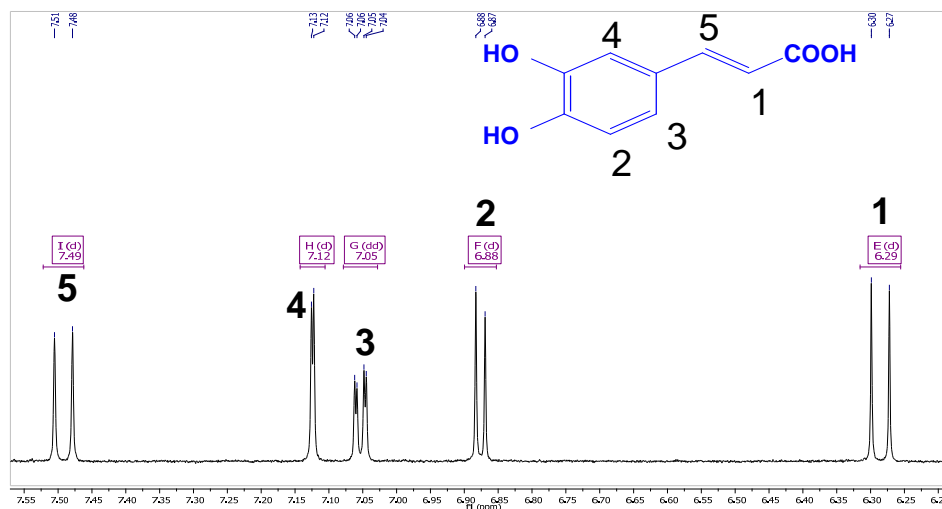


Proton NMR: Caffeic acid

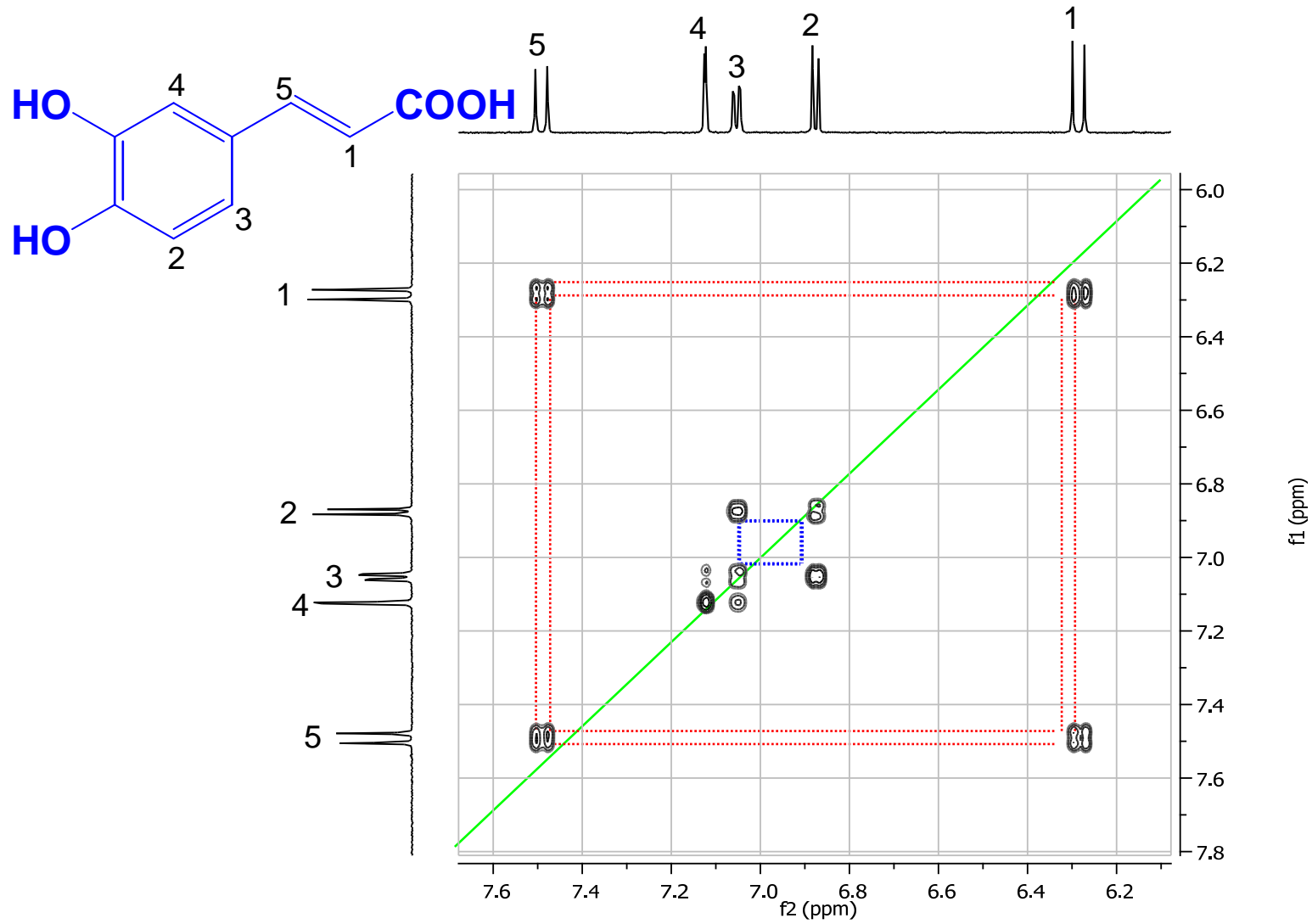


1H-NMR: Potato Extract

potato extract



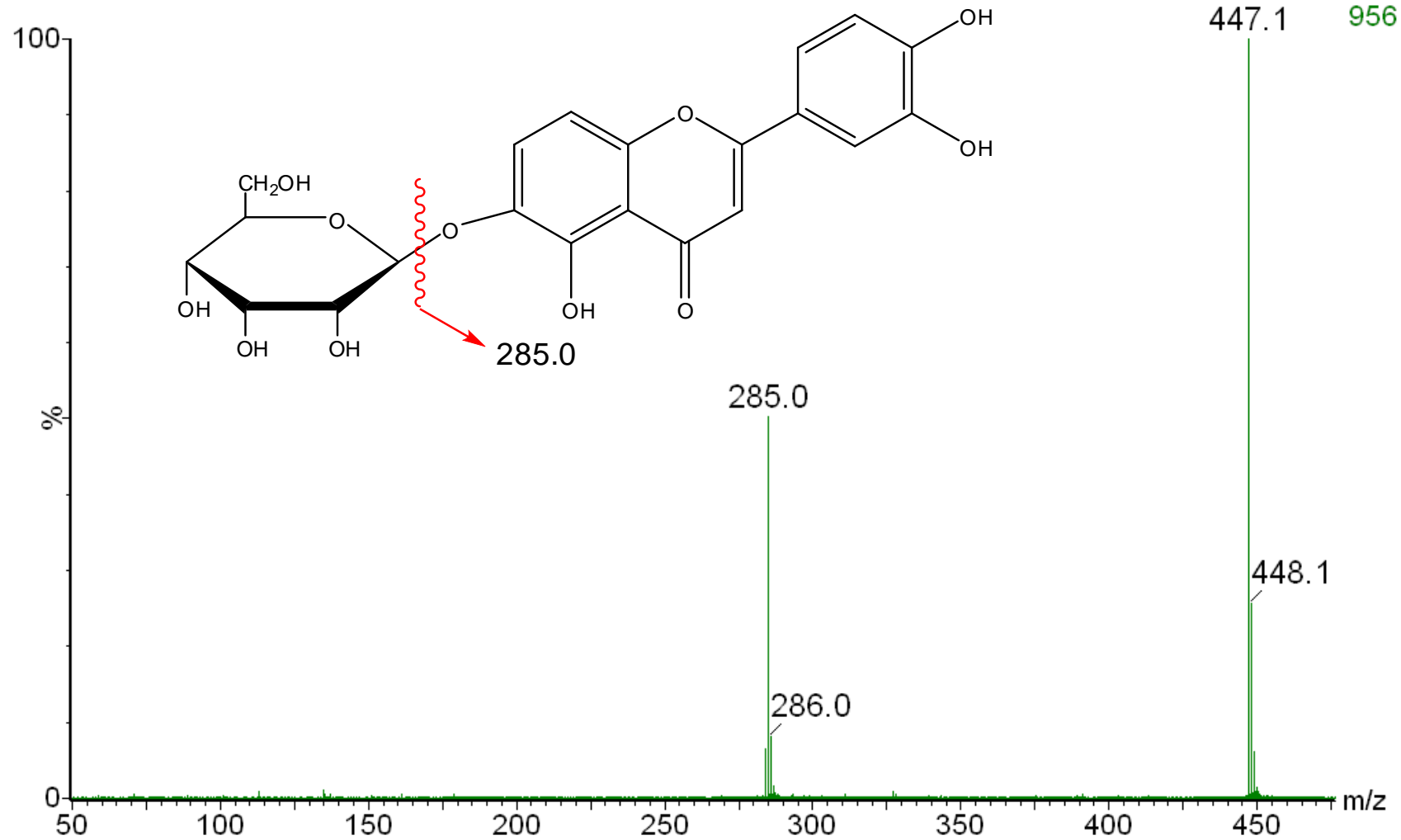
2D NMR (COSY): Caffeic acid



MS/MS of Luteolin-7-O-glucoside

DILIP473 41 (8.866)

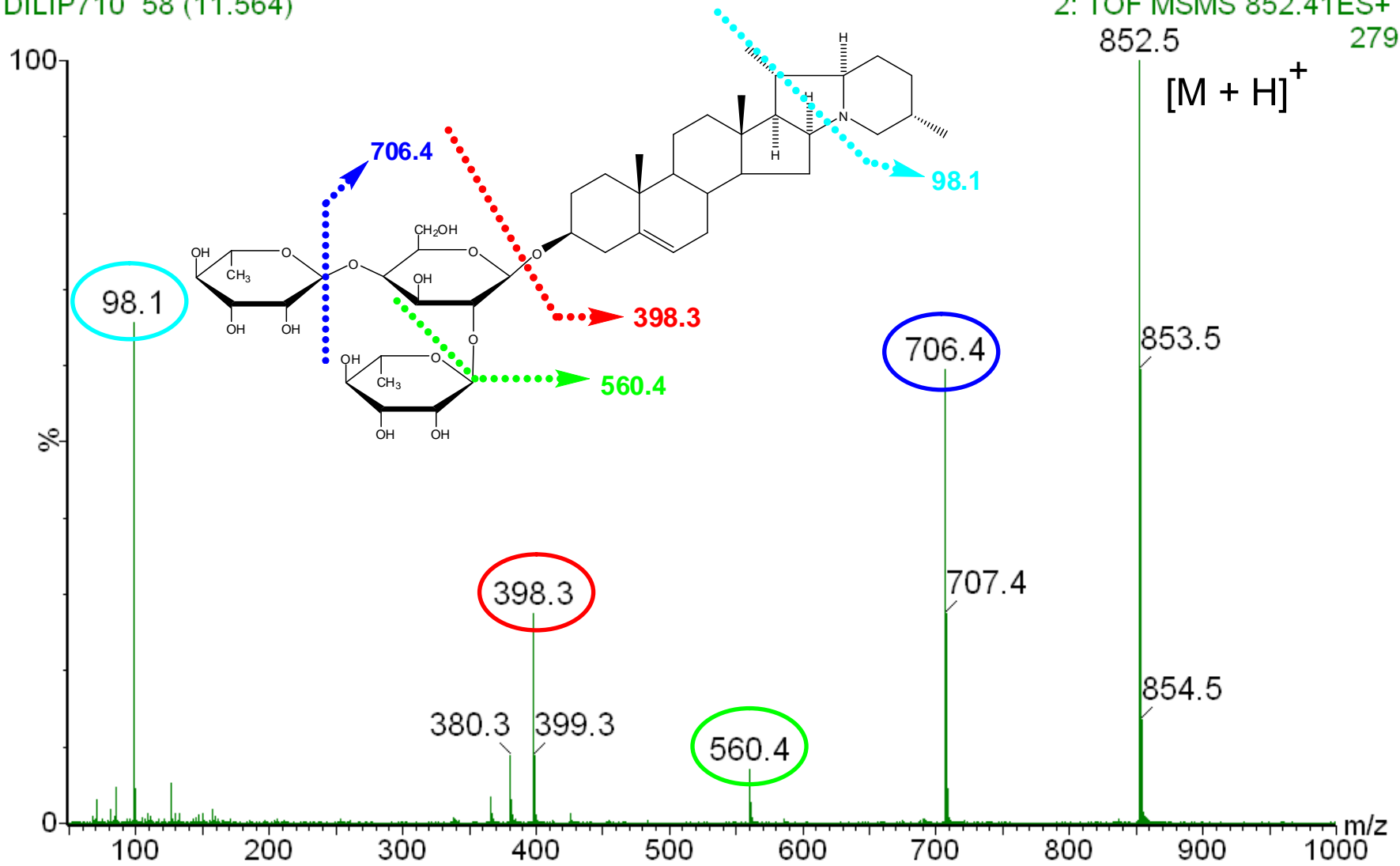
1: TOF MSMS 447.07ES-



MS/MS OF α -Chaconine

DILIP710 58 (11.564)

2: TOF MSMS 852.41ES+
852.5 279



Accurate masses of glycoalkaloid components

Analyte	Calculated	Observed	Error (ppm)
a-chaconine	852.5109	852.5108	0.1
- 1 sugar	706.4530	706.4548	2.5
- 2 sugars	560.3951	560.3950	0.2
Solanidine	398.3423	398.3408	3.8

Results



> 30 polyphenols identified in crude extracts.

MS/NMR



2 polyphenols & 5 glycoalkaloids confirmed; project ongoing.



Confirmed chemical structures of 3 polacetylenes.

Acknowledgements

Anna Gadaj/Hilde;
Ashish Rawson/Tassos Koidis;
Mohammad B. Hossain.

Padraig McLoughlin
Jimmy Muldoon (UCD CSCB)
Nigel Brunton.

CONCLUSION

