



SAPIENZA
UNIVERSITÀ DI ROMA

Discrimination and structural characterization of protonated forms of two isomeric flavonoids by IRMPD spectroscopy, IMS, CID-MS, and computational approaches

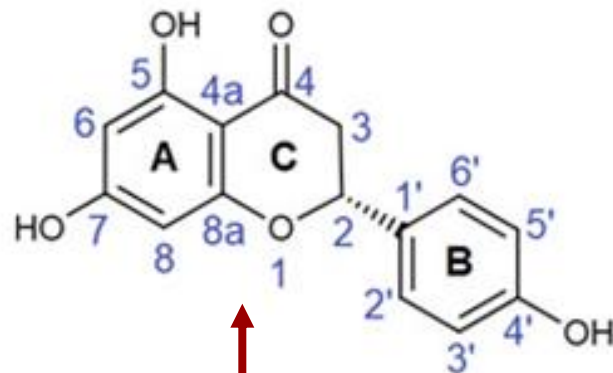
Department of Pharmaceutical Chemistry and Technology, Sapienza, University of Rome

Lucretia Rotari, Davide Corinti, Maria Elisa Crestoni, Simonetta Fornarini and Barbara Chiavarino



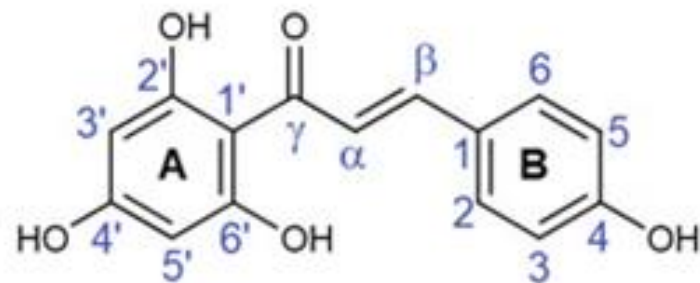
Naringenin and Naringenin Chalcone

Flavonoids are class of **natural products**. They belong to a class of plant secondary metabolites having a **polyphenolic structure**. Flavonoids are associated with a broad spectrum of **health-promoting effects**.



*alkaline and
neutral media*

CHI



Naringenin (Nar)

➤ Present in plants of the genus *Citrus*

➤ *Activities*: anti-inflammatory, antibacterial, antiviral, anticancer and antioxidant



Naringenin Chalcone (ChNar)

➤ Present in the plants of the family Compositae, Lamiaceae and Solanaceae

➤ *Activities*: anti-inflammatory and antiallergic



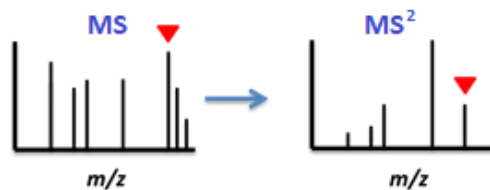
Tsao, R. *Nutrients*. **2010**, 2, 1231–1246.

Aim of the work:

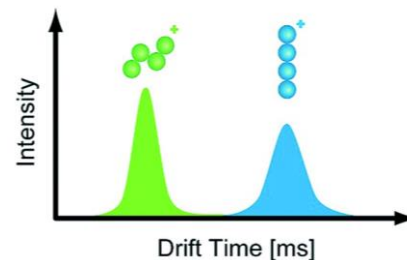
1. To determine structural features and conformational distribution of protonated Nar and ChNar isolated in the gas phase
2. To investigate the nature of the metabolites, present in methanolic extracts of commercial tomatoes and grapefruits

Methodology:

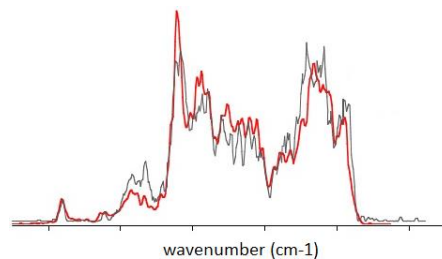
- CID measurements



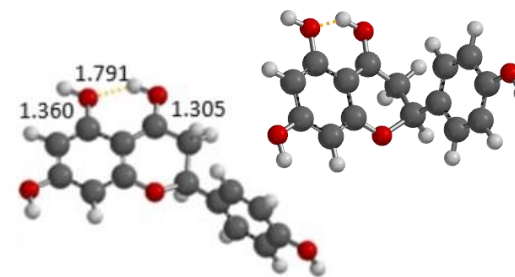
- Ion Mobility – Mass spectrometry



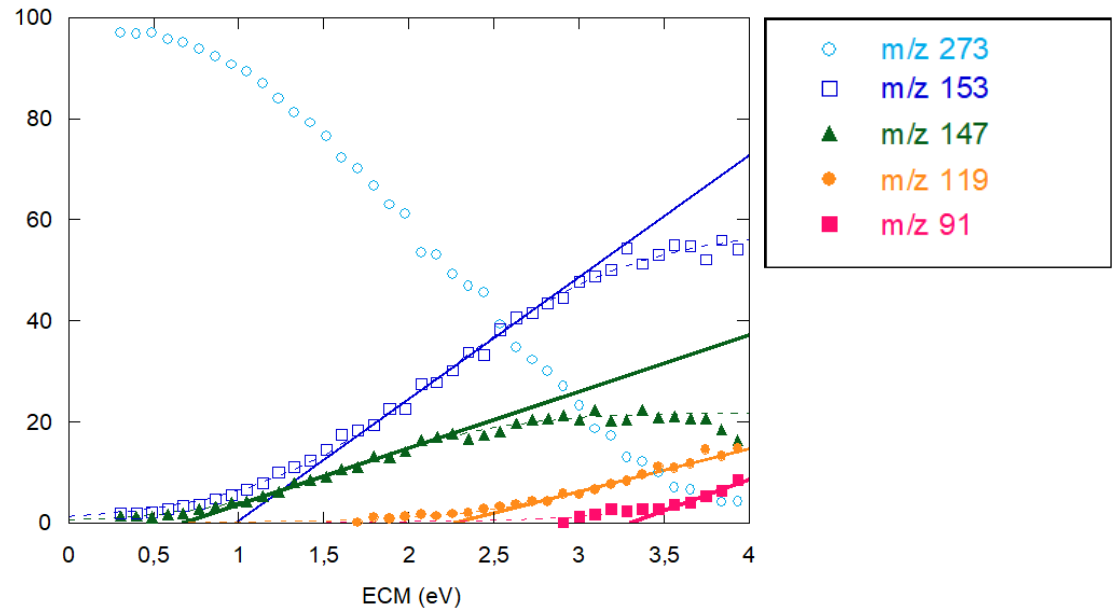
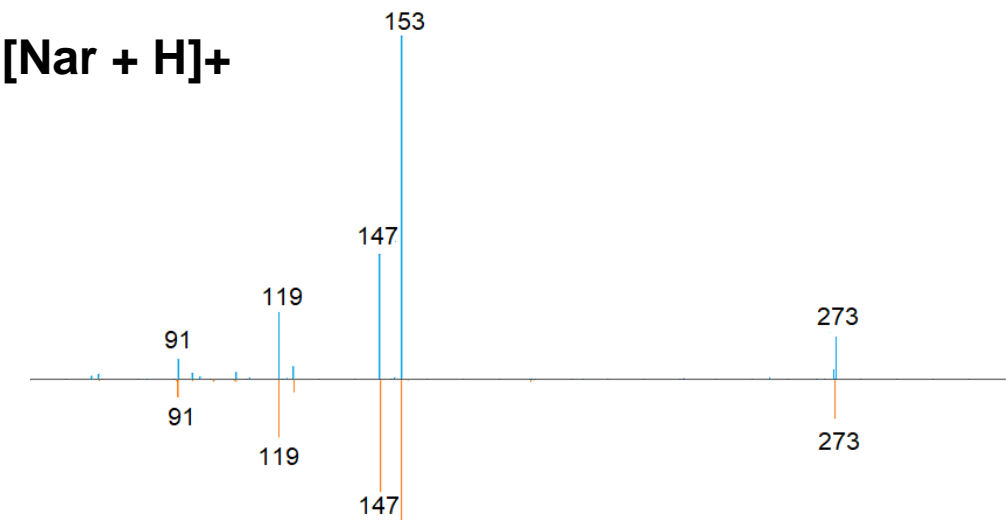
- IRMPD spectroscopy



- DFT calculations at the B3LYP/6-311++G(d,p) level of theory

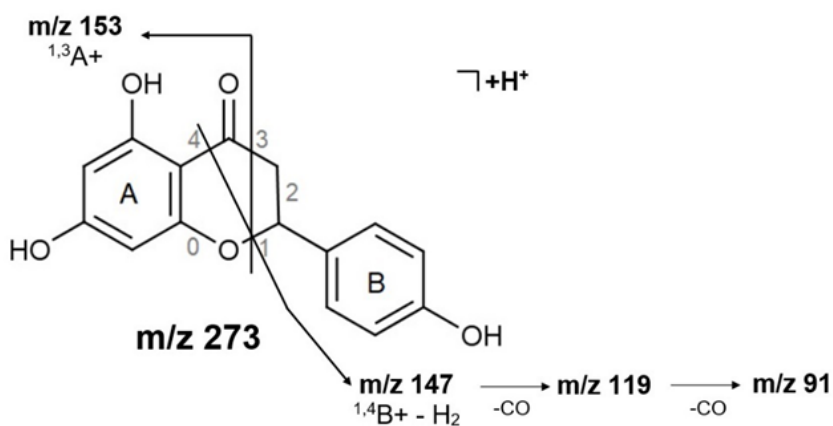


CID experiments



[ChNar + H]⁺

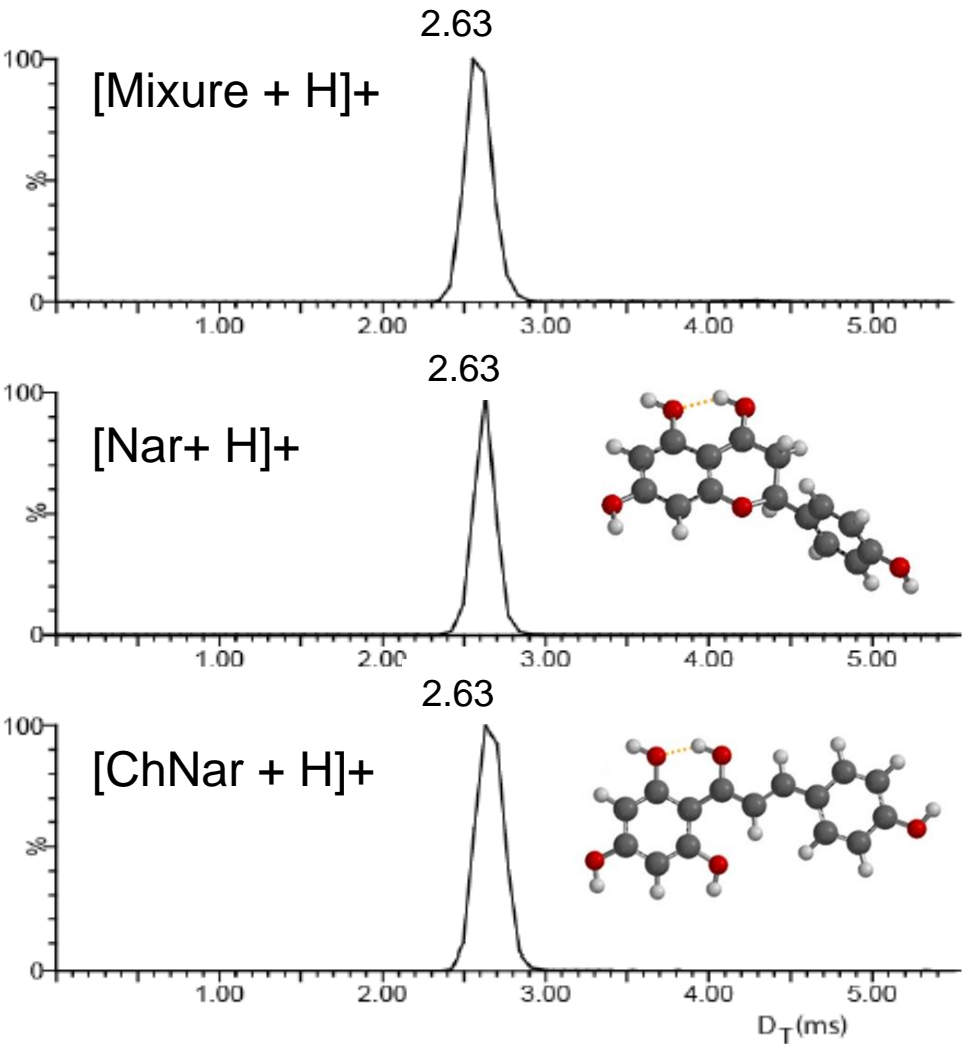
Comparison of CID mass spectra of [Nar+H]⁺ and [ChNar+H]⁺ (in blue and orange respectively), recorded at CE of 35 eV. Fragmentation pathways of protonated naringenin.



Fragment ion (m/z)	TE (eV) of [Nar+H] ⁺	TE (eV) of [ChNar+H] ⁺
153	1.00	1.15
147	0.70	1.00
119	2.30	2.50
91	3.30	3.60

Phenomenological threshold energies (TEs)

Ion Mobility experiments

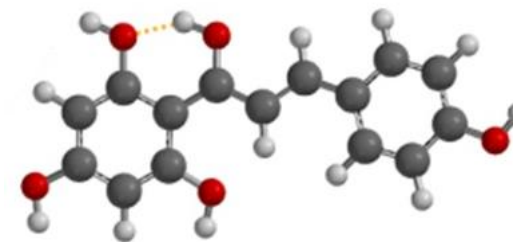
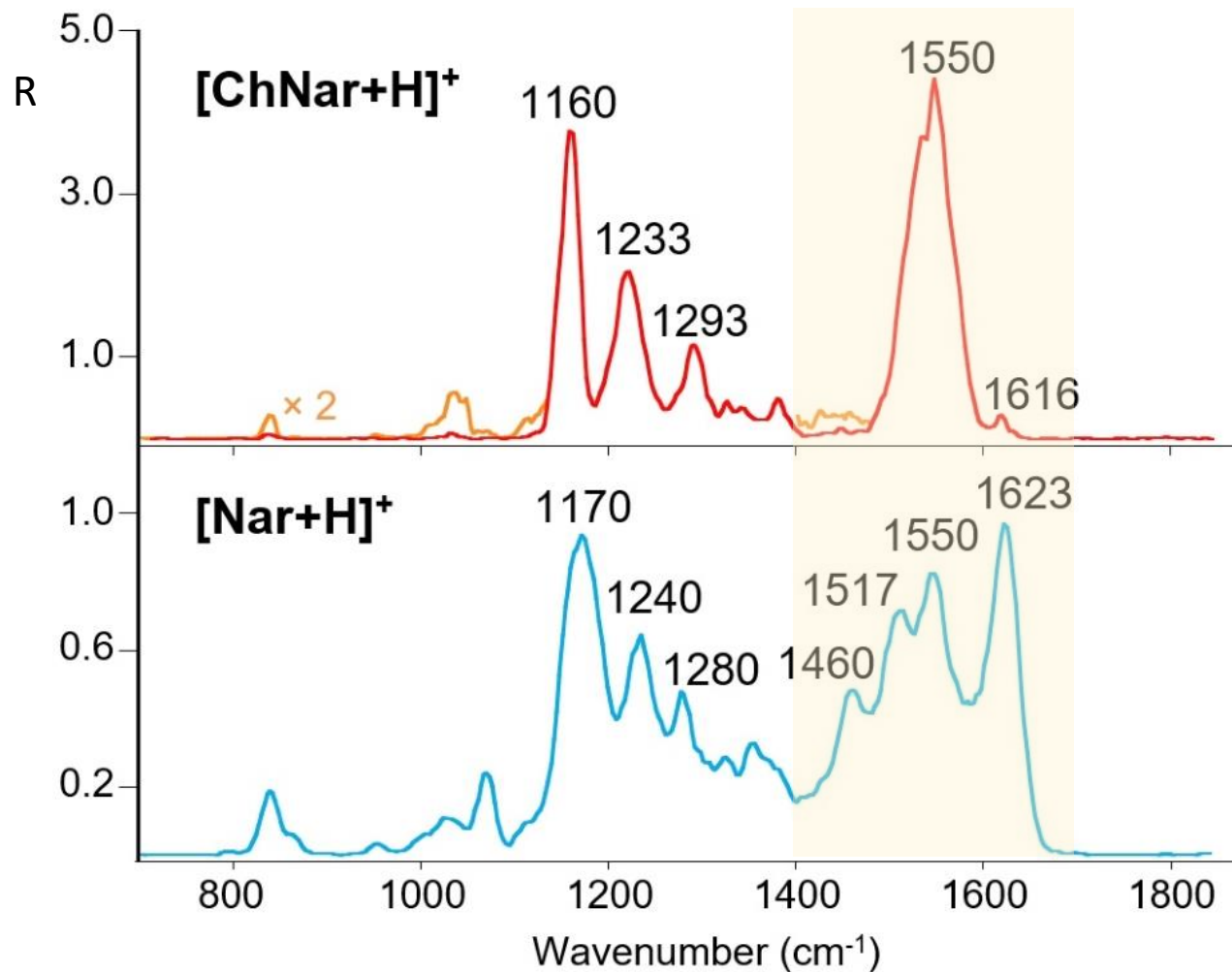


Extracted mobilogram of protonated species observed at m/z 273.

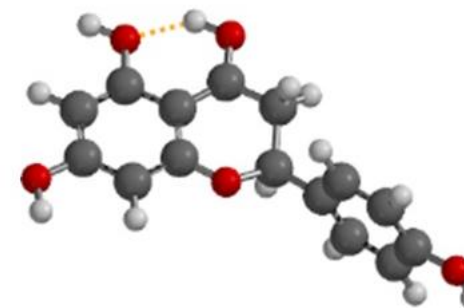
Compound	CCS exp. (Å ²)	CCS calculated (Å ²)
$[Nar+H]^+$	160.9	160.8
$[ChNar+H]^+$	160.9	160.1

Extrapolated and calculated CCS of protonated species.

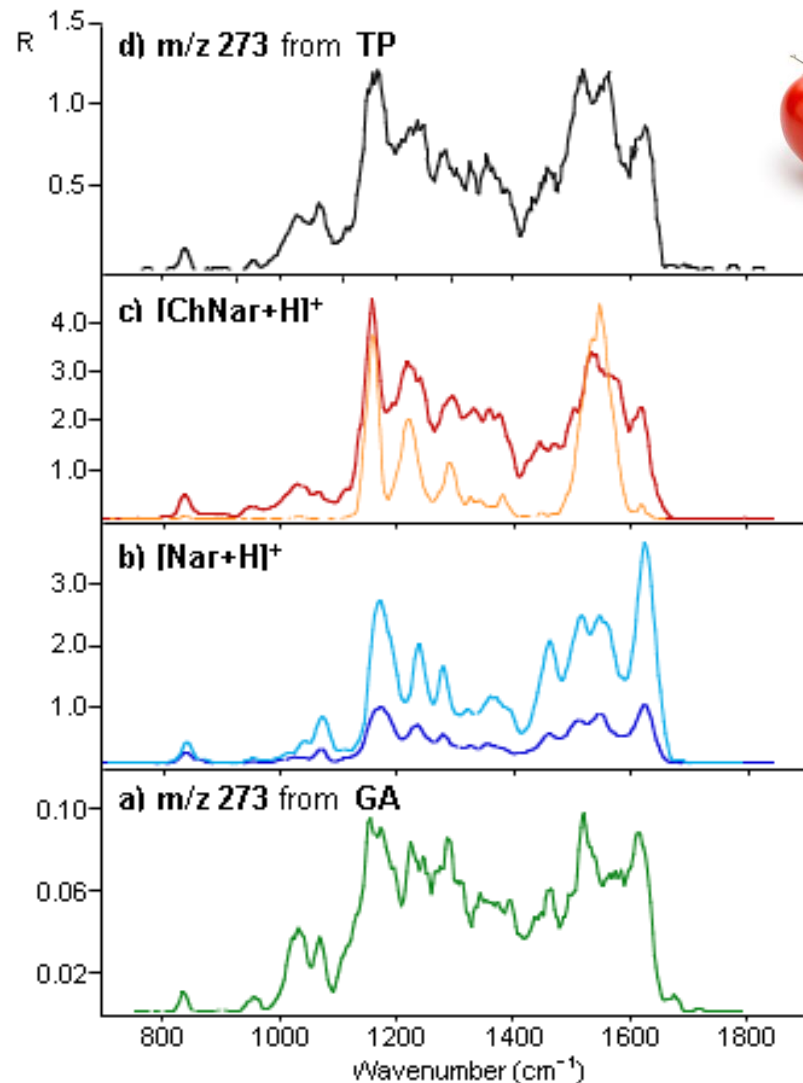
IRMPD experiments



Comparison between the IRMPD spectra of protonated Naringenin (blue trace) and Naringenin Chalcone (red and orange trace).



IRMPD spectra of methanolic extracts



IRMPD spectrum of the ion m/z 273 isolated from the tomato peel extract (black profiles) and albedo grapefruit extract compared with the IRMPD spectra of the standard compounds, $[ChNar+H]^+$ (blue panel) and $[Nar+H]^+$ (red panel).

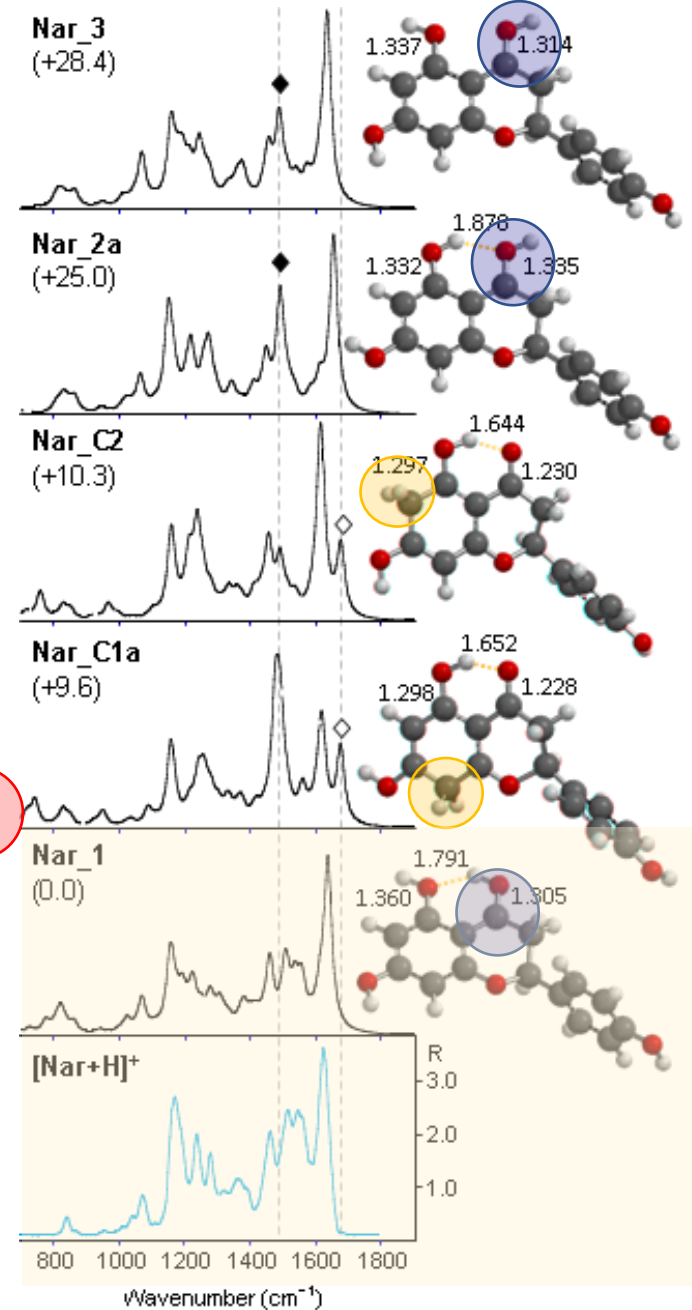
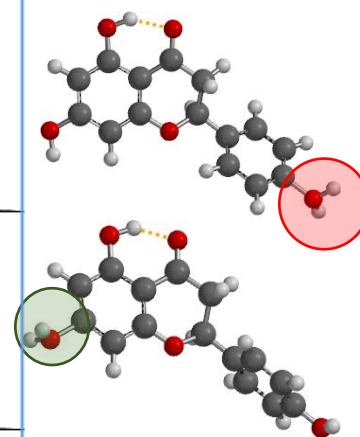
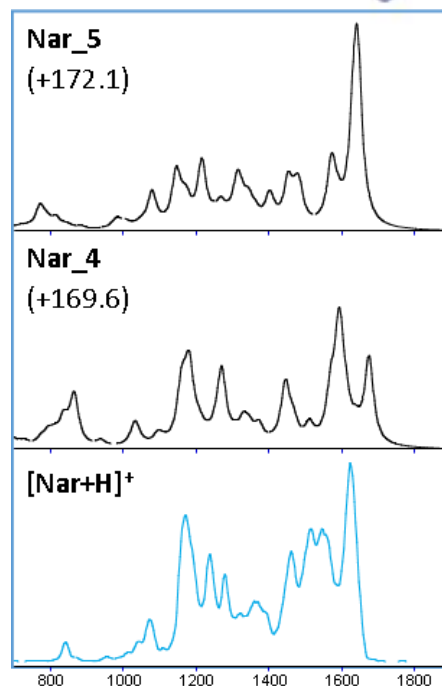
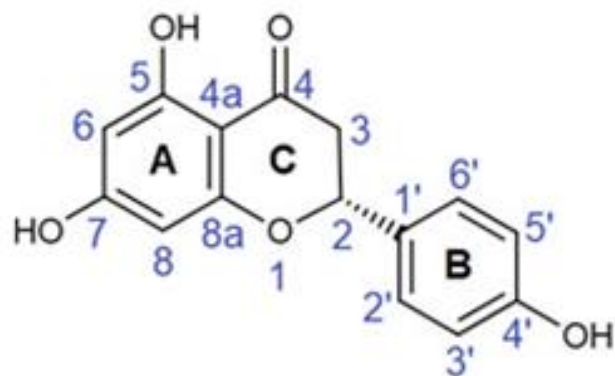


IRMPD spectrum and the calculated IR spectra of isomers of $[\text{Nar} + \text{H}]^+$

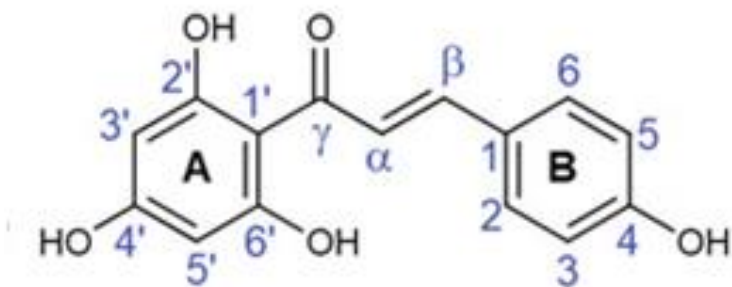
IRMPD spectrum of $[\text{Nar} + \text{H}]^+$ (blue trace) compared with calculated IR spectra of **Nar_1**, **Nar_C1a**, **Nar_C2**, **Nar_2a**, **Nar_3**, **Nar_4** and **Nar_5** whose optimized structures are on the right.

An open diamond \diamond indicates the calculated $\text{C}4=\text{O}$ stretching mode at 1676 cm^{-1} for **Nar_C1a**, **Nar_C2** and a black diamond \blacklozenge indicates the $\text{C}5\text{-OH}$ stretching mode expected at 1490 cm^{-1} for **Nar_2a** and **Nar_3**.

B3LYP/6-311++G(d,p)

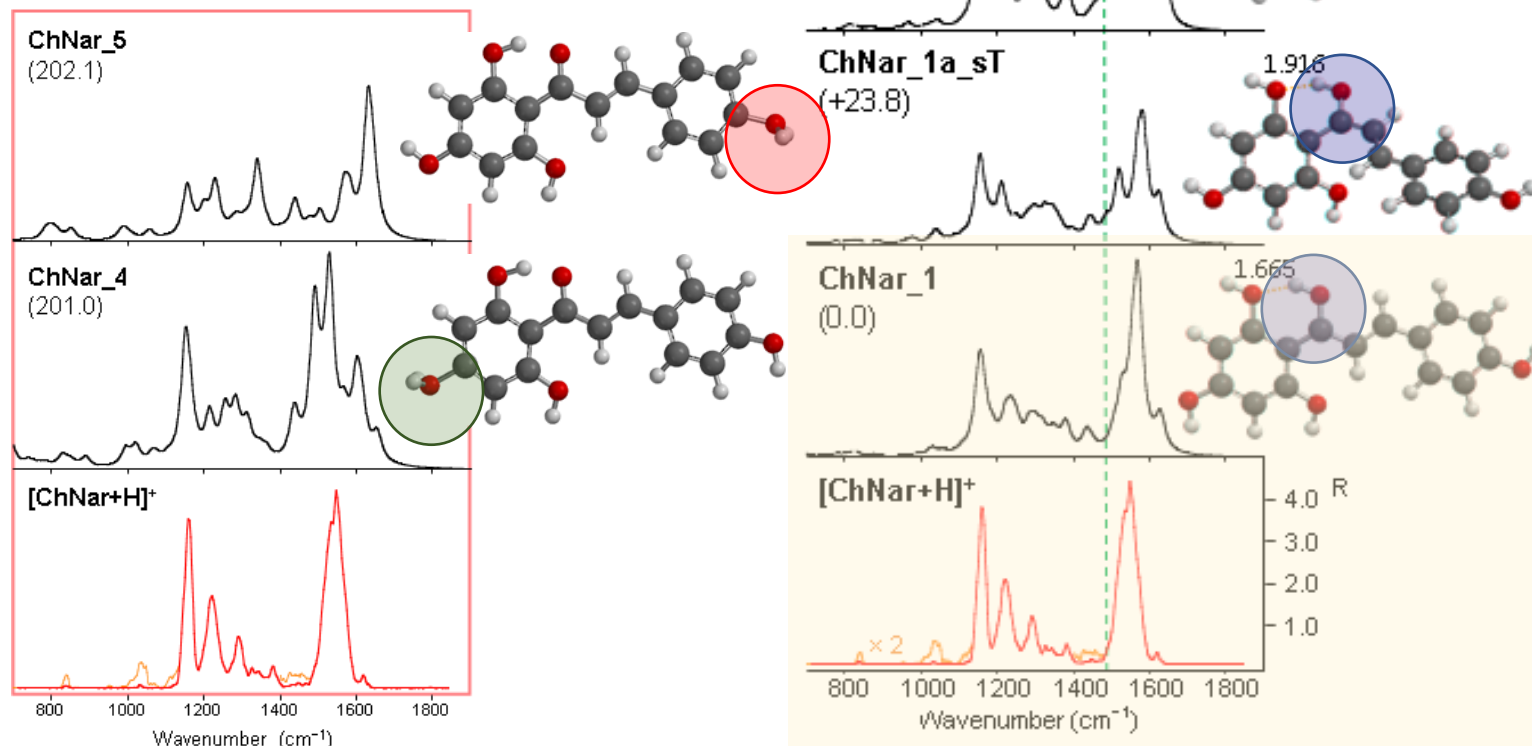


IRMPD spectrum and the calculated IR spectra of isomers of $[\text{ChNar} + \text{H}]^+$



IRMPD spectrum of $[\text{ChNar} + \text{H}]^+$ (red trace) compared with calculated IR spectra of **ChNar_1**, **ChNar_1a_sT**, **ChNar_2a**, **ChNar_3a**, **Nar_3**, **ChNar_4** and **ChNar_5** whose optimized structures are on the right.

B3LYP/6-311++G(d,p)



Conclusions

- CID and IMS experiments hardly differentiate the two isomers, while IRMPD spectroscopy turned out to as an efficiently method in distinguishing naringenin from its chalcone.
- IRMPD spectroscopy is a powerful tool in contemporary targeted and conformational analysis of food metabolites, as confirm the present results.
- Comparisons between experimental IRMPD and calculated IR spectra provided the geometries adopted by the two protonated ions in the gas phase.

ACKNOLEDGMENTS



Prof.ssa Simonetta Fornarini
Prof.ssa Maria Elisa Crestoni
Prof.ssa Barbara Chiavarino
PhD Davide Corinti
Dott. Alba Lasalvia



Pr. Aura Tintaru



Pr. Jos Oomens

*Thank you for
your attention!*



*European Network of Fourier-Transform Ion-
Cyclotron-Resonance Mass Spectrometry Centers*

lucretia.rotari@uniroma1.it