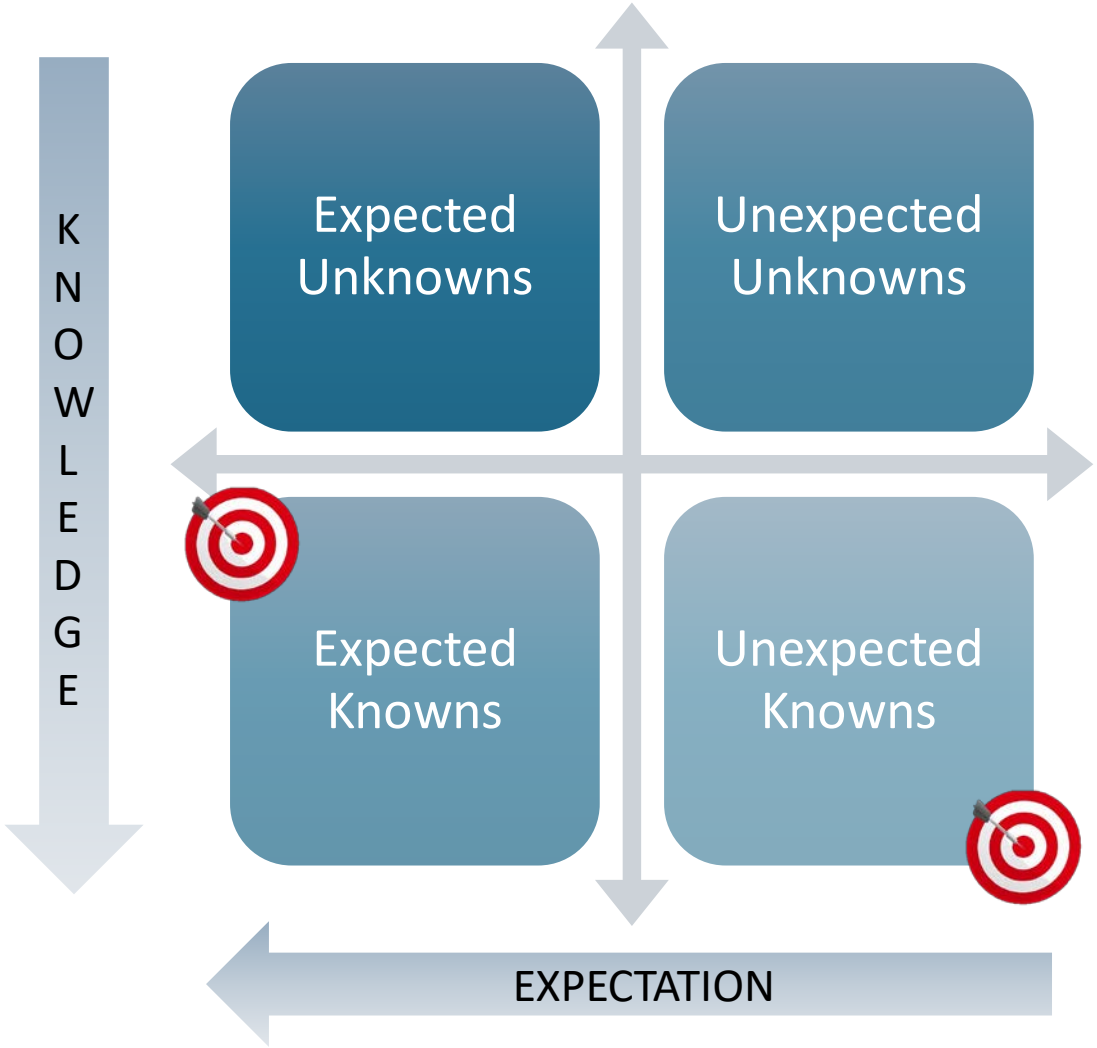
UNIVERSITÀ DI PARMA

Innovations in the detection of unknown contaminants by non-targeted MS

Chiara Dall'Asta

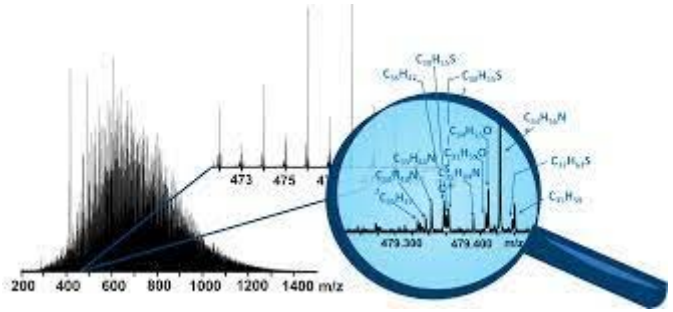
Dealing with unknowns in food analysis

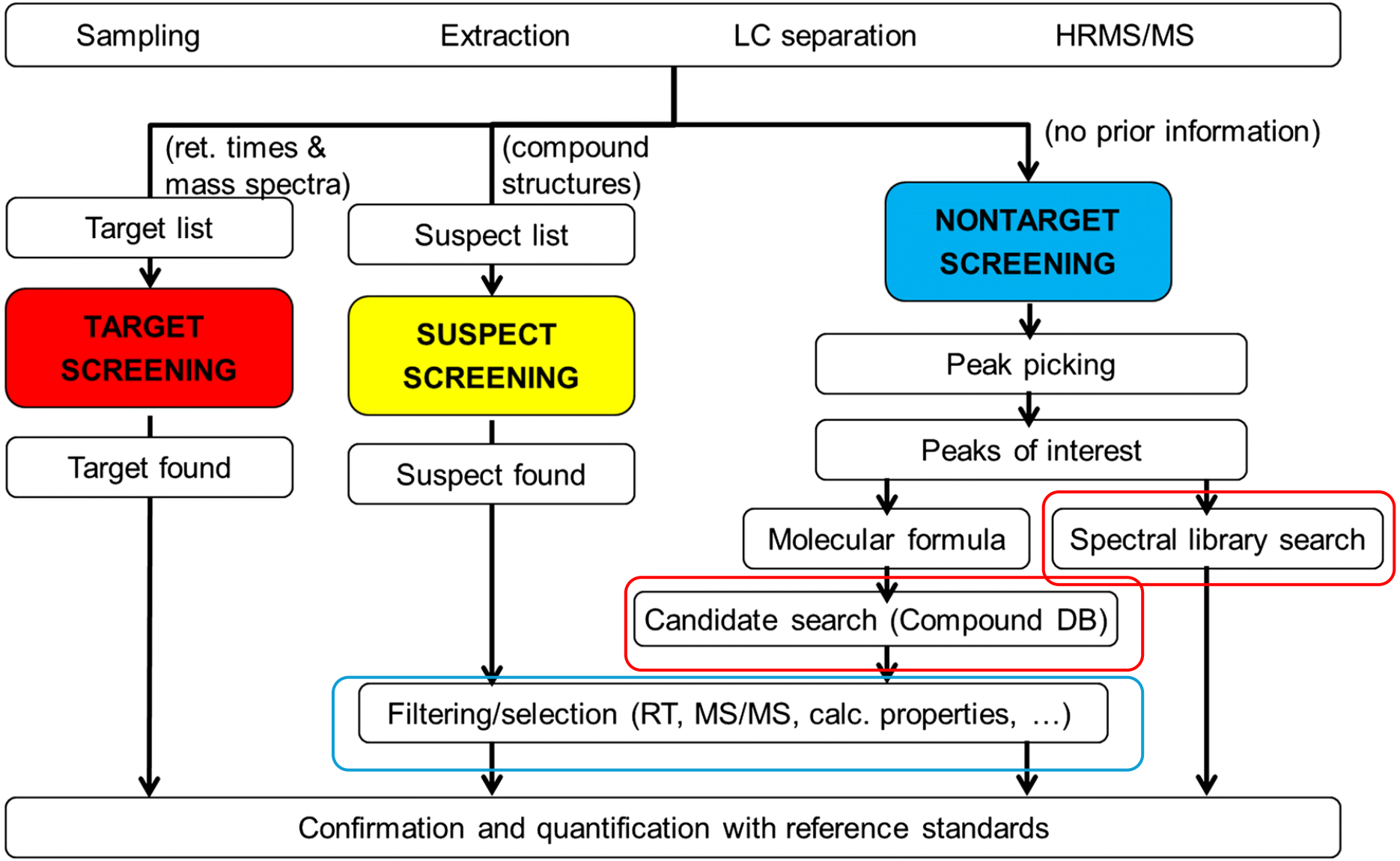


1. Triple Quad MS
2. High Res MS

(orbitrap versus QTOF)

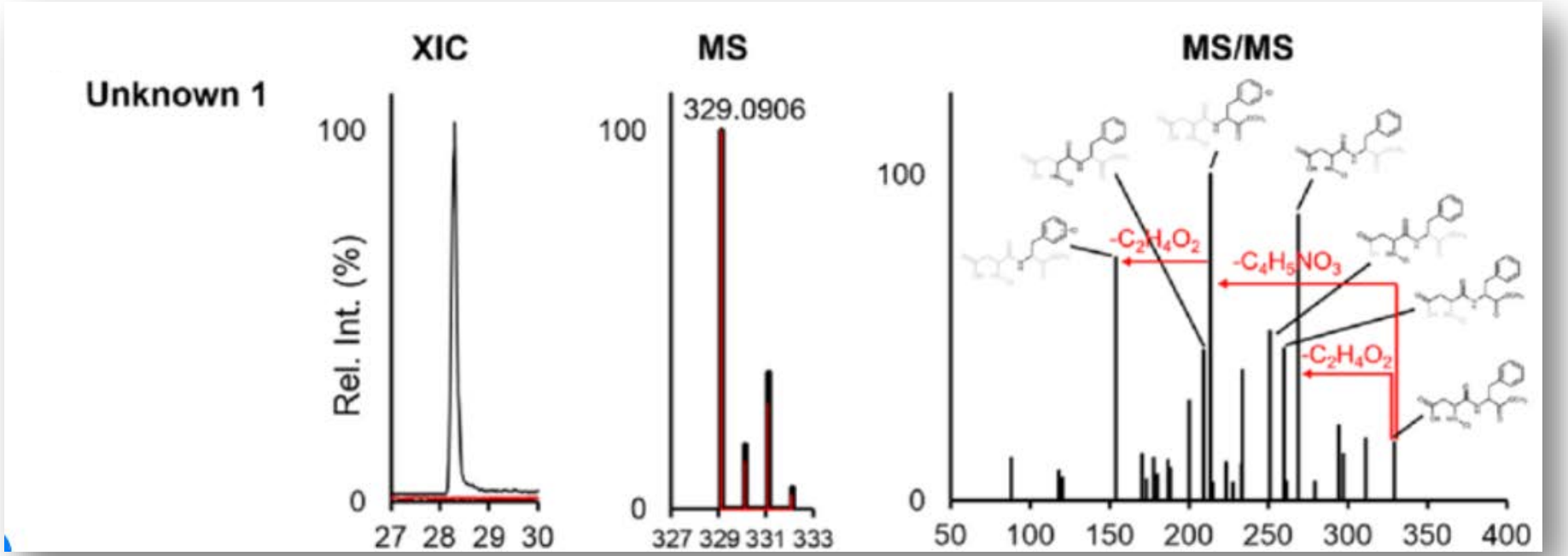
3. Ambient MS





Brack et al. 2019

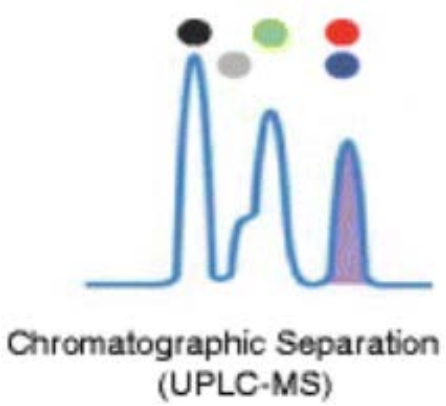
Dealing with unknowns in food analysis

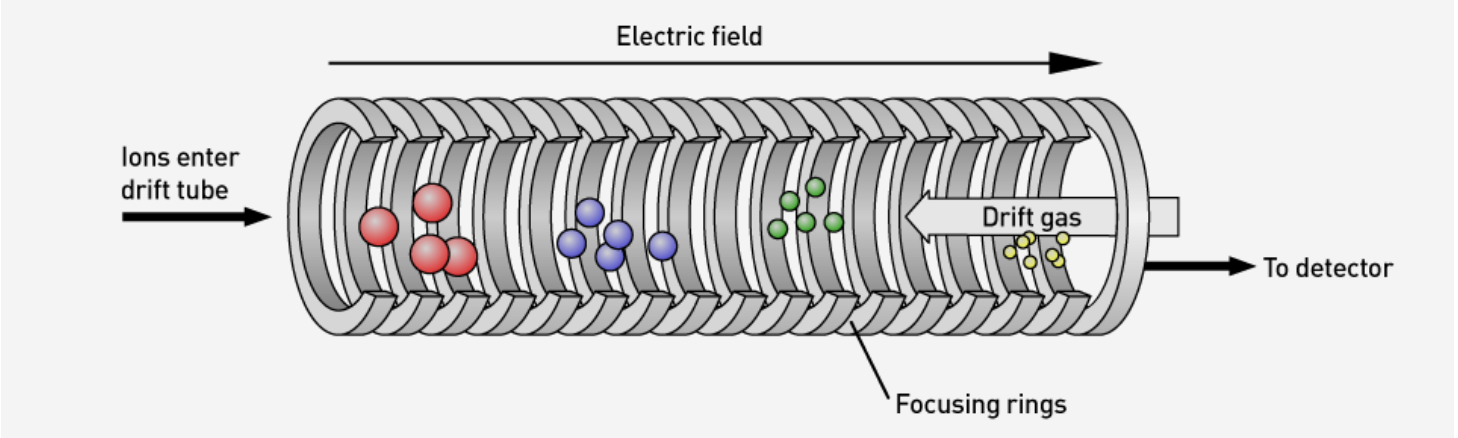


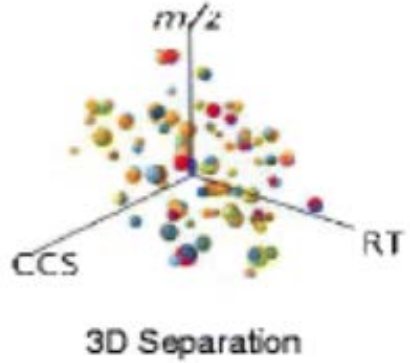
- Ion mobility technology
- Machine learning and other advanced chemometrics techniques
- Open source tool for compound discovery/annotation
- Comprehensive database

Ion Mobility – Adding a new dimension to HR-MS

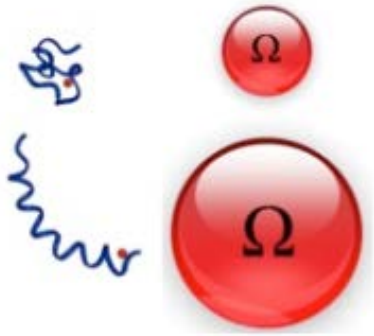
IM-MS allows the separation of ionized molecules based on their structural properties such as size and shape, in addition to their mass-to-charge ratio.







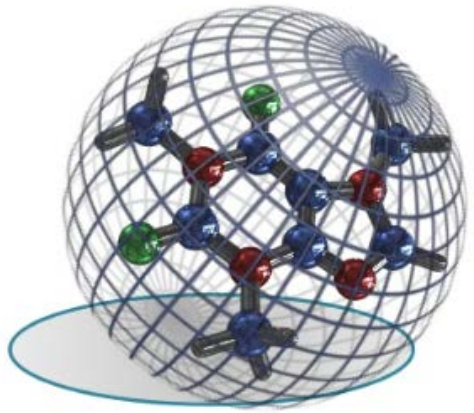
The time it takes for the charged species to transverse the drift tube can then be converted into a collision cross section value, which is representative of a rotationally averaged surface area.



Ion Mobility – Adding a new dimension to HR-MS

- Collision Cross Section (CCS) value is a robust and precise physicochemical property of an ion wich is related to its chemical structure and three-dimensional conformation.

- CCS values can be estimated computationally if the 3D structure is known,



Theoretical CCS collection

Ab initio calculation

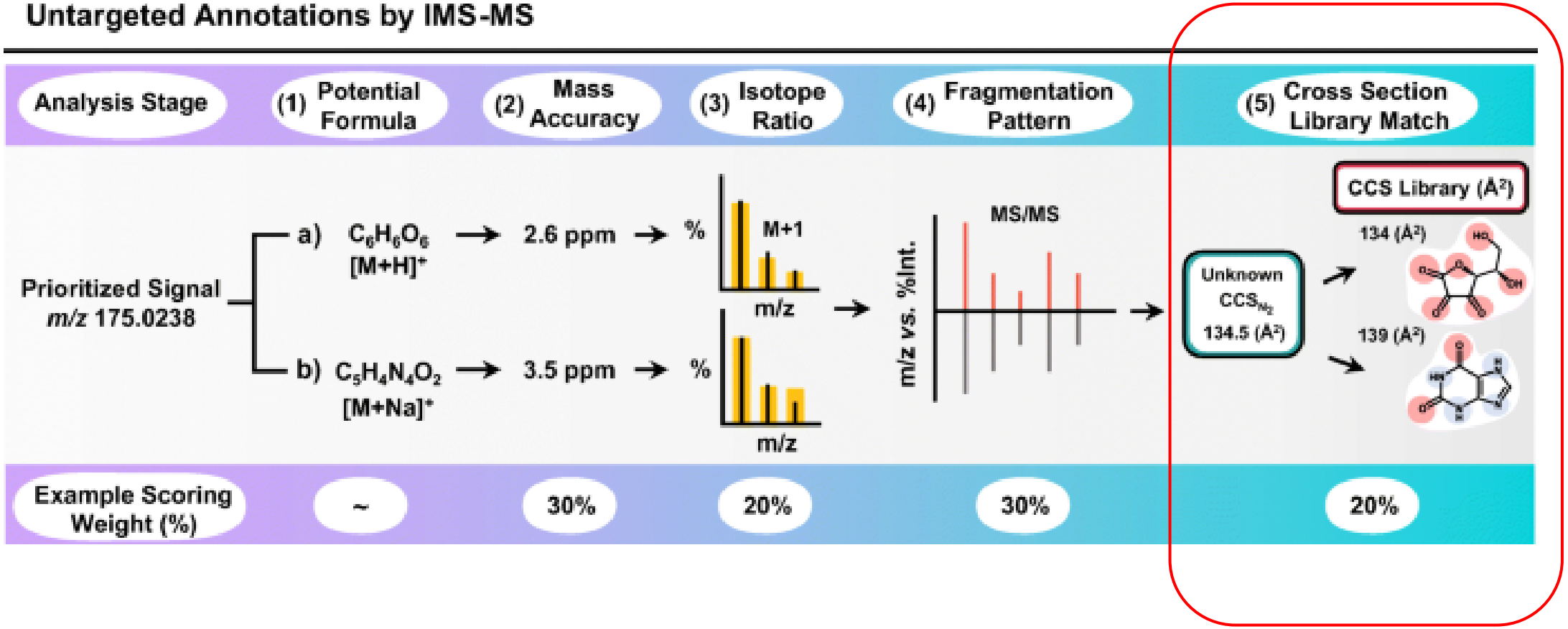
MOBCAL-MPI 2.0

Machine Learning

AllCCS

DeepCCS

Workflow of untargeted identifications by IMS-MS



Dodds & Baker, JASMS, 2019

Ion Mobility – Challenges and Limitations

Different technologies

Different calibration protocols

Different algoritms for in silico generation of CCS

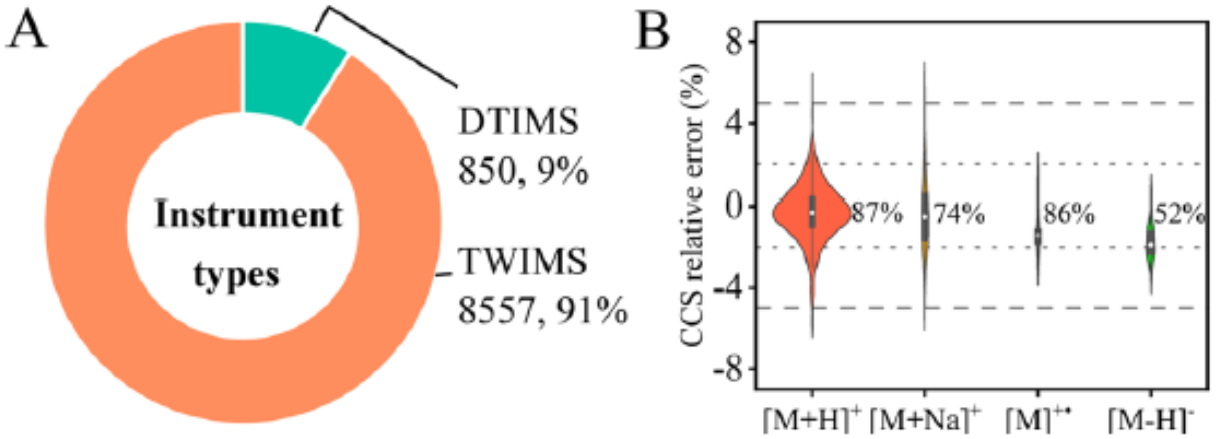
Gaps in DB population

Song et al. 2023, Environ.Sci.Technol.

RECOMMENDATIONS FOR REPORTING ION MOBILITY MASS SPECTROMETRY MEASUREMENTS

Valérie Gabelica,[1]* Alexandre A. Shvartsburg,[2]** Carlos Afonso,[3] Perdita Barran,[4] Justin L.P. Benesch,[5] Christian Bleiholder,[6] Michael T. Bowers,[7] Aivett Bilbao,[8] Matthew F. Bush,[9] J. Larry Campbell,[10] Iain D.G. Campuzano,[11] Tim Causon,[12] Brian H. Clowers,[13] Colin S. Creaser,[14] Edwin De Pauw,[15] Johann Far,[15] Francisco Fernandez-Lima,[16] John C. Fjeldsted,[17] Kevin Giles,[18] Michael Groessl,[19] Christopher J. Hogan Jr,[20] Stephan Hann,[12] Hugh I. Kim,[21] Ruwan T. Kurulugama,[17] Jody C. May,[22] John A. McLean,[22] Kevin Pagel,[23] Keith Richardson,[18] Mark E. Ridgeway,[24] Frédéric Rosu,[25] Frank Sobott,[26,27,28] Konstantinos Thalassinos,[29,30] Stephen J. Valentine,[31] and Thomas Wyttenbach[7]

Mass Spec Rev. 2019



How to deal with unexpected contaminants in the real life?

BOTANICALS AND HERBAL SUPPLEMENTS

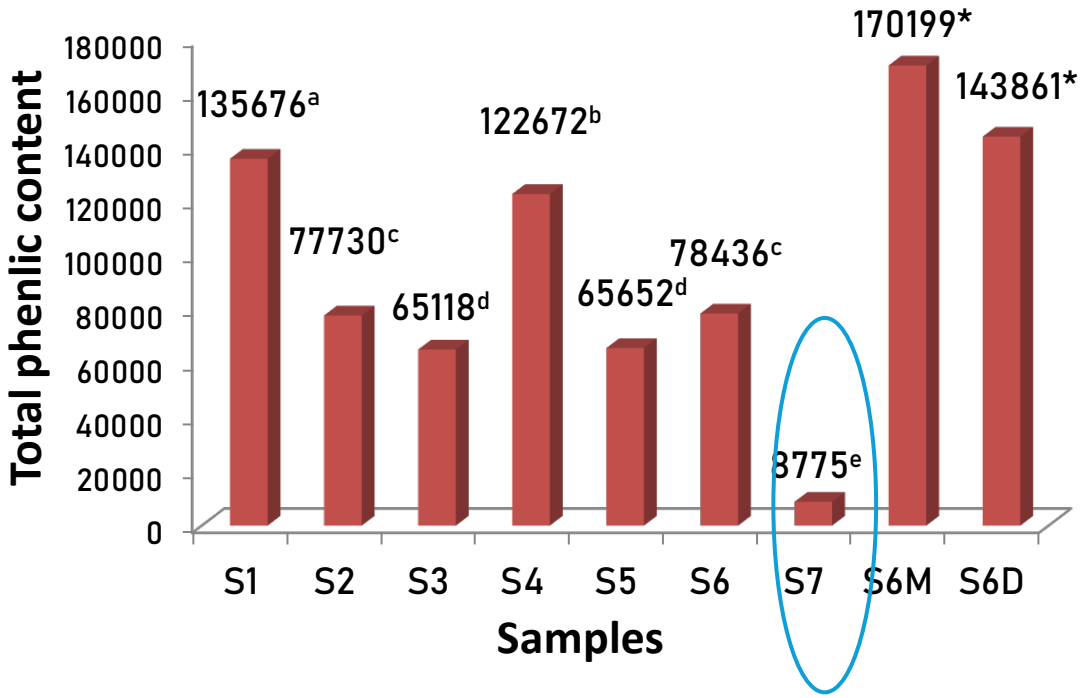
1. **LACK OF AN HARMONISED** DEFINITION ACROSS COUNTRIES AND **HIGH VARIABILITY** OF THE MANDATORY REQUIREMENTS FOR COMMERCIALISATION

2. STRONG INCREASE OF THE ONLINE MARKET

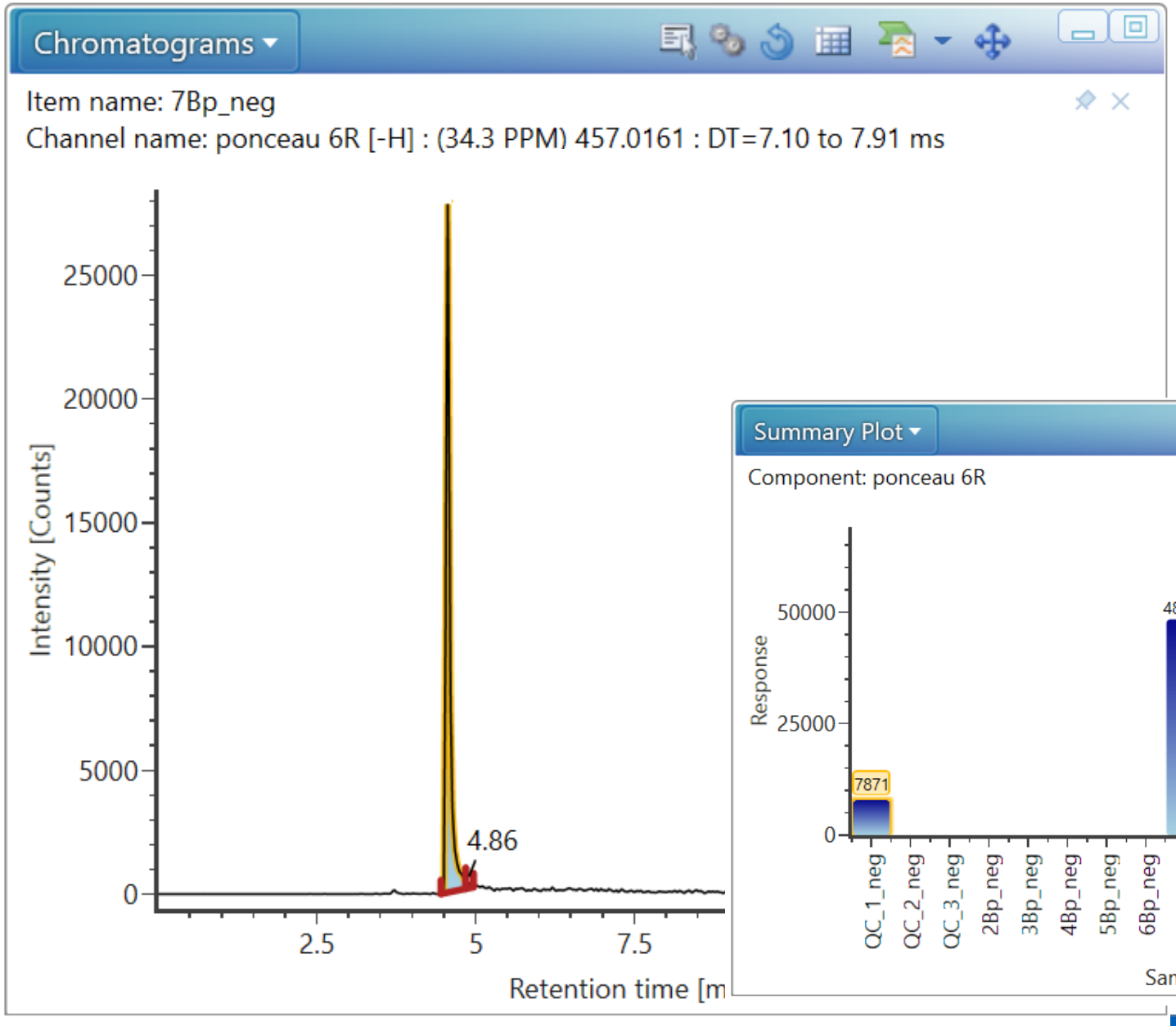
CASE STUDY 1 – Unexpected dyes in sumac powder

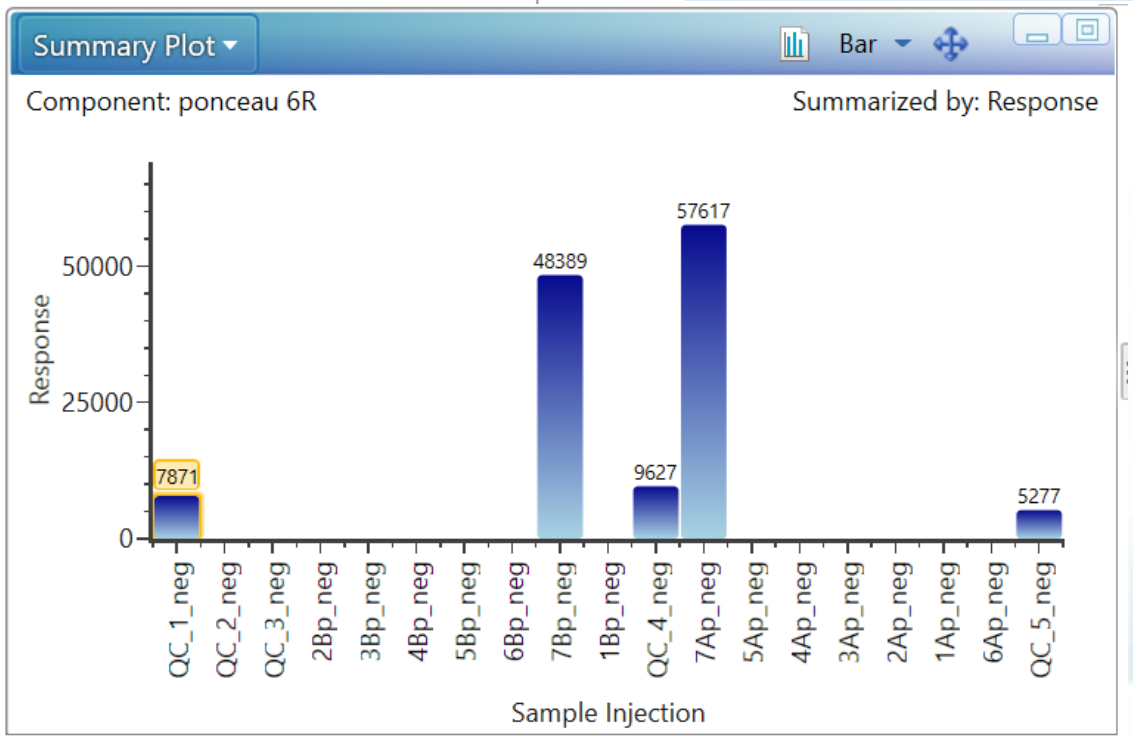
Rhus coriaria

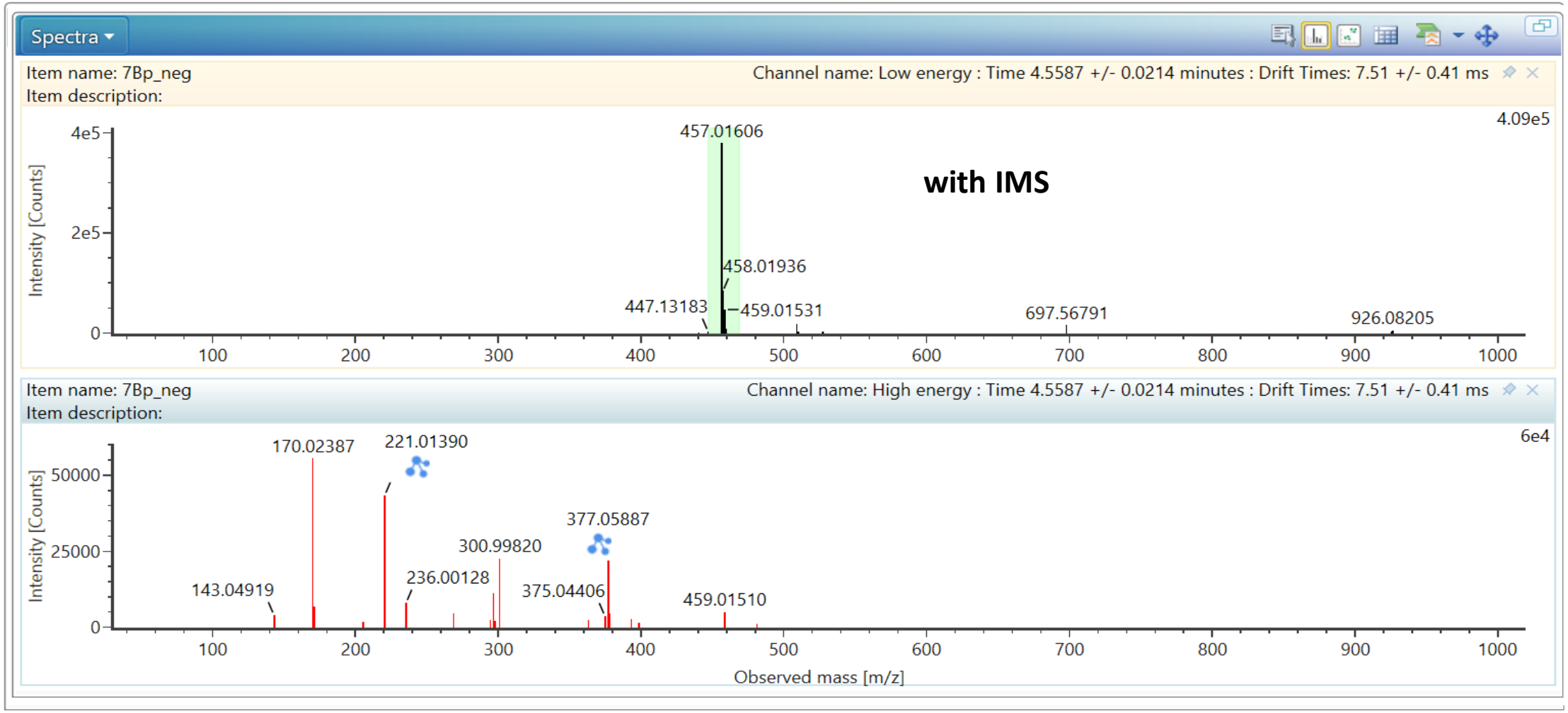
Cultivated in the Mediterranean Basin
Traditionally used as a spice and in traditional medicine

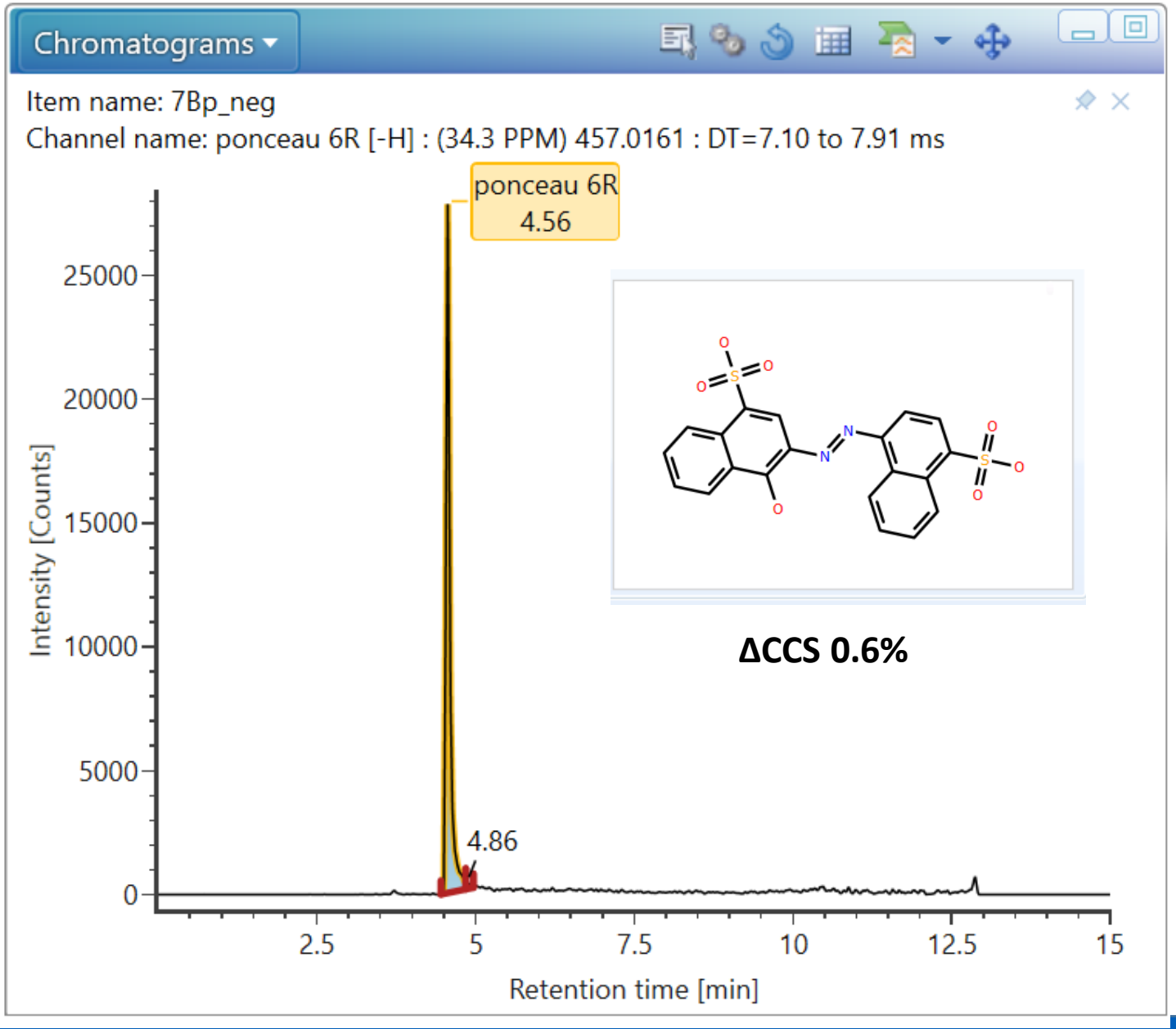


RETROSPECTIVE ANALYSIS and USE of UNIFI CURATED LIBRARY for ADULTERANTS






Spectra
Item name: 7Bp_neg
Item description:
Channel name: Low energy : Time 4.5587 +/- 0.0214 minutes : Drift Times: 7.51 +/- 0.41 ms
4.09e5
4e5
2e5
0
Intensity [Counts]
457.01606
458.01936
447.13183
459.01531
697.56791
926.08205
with IMS
100
200
300
400
500
600
700
800
900
1000
Item name: 7Bp_neg
Item description:
Channel name: High energy : Time 4.5587 +/- 0.0214 minutes : Drift Times: 7.51 +/- 0.41 ms
6e4
50000
25000
0
Intensity [Counts]
170.02387
221.01390
377.05887
300.99820
236.00128
143.04919
375.04406
459.01510
Observed mass [m/z]



ΔCCS 0.6%

USE of UNIFI CURATED LIBRARY for ADULTERANTS

Filtering by mass accuracy (< 5 ppm) and by CCS (2%)

CASE STUDY 2 – Adulterants in Red Yeast Rice

Traditionally obtained by fermentation with *Monascus* spp. fungal starters. Fermentation leads to the species-dependent accumulation of monacolins and pigments

2011 EFSA HEALTH CLAIM
"Monacolin K from RYR contributes to the maintenance of normal blood cholesterol concentrations"

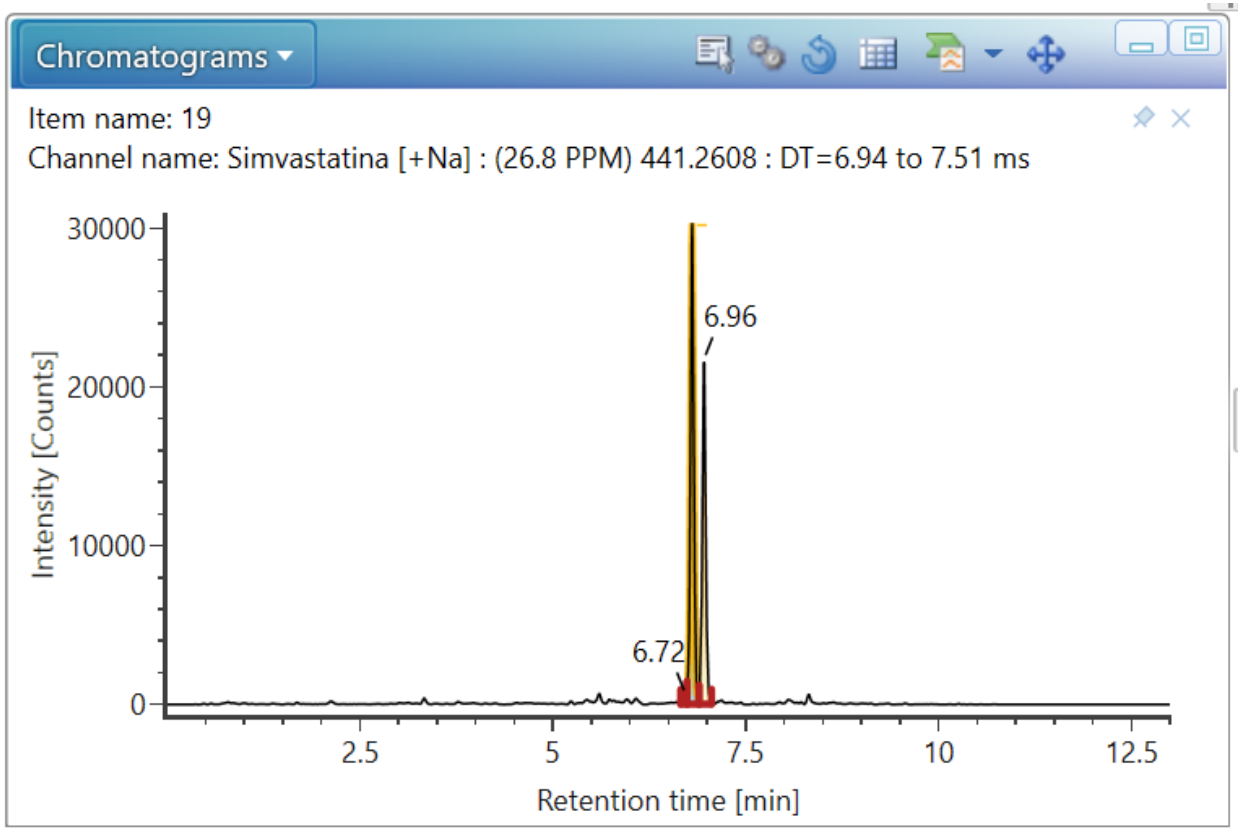
10 mg/die of MK required

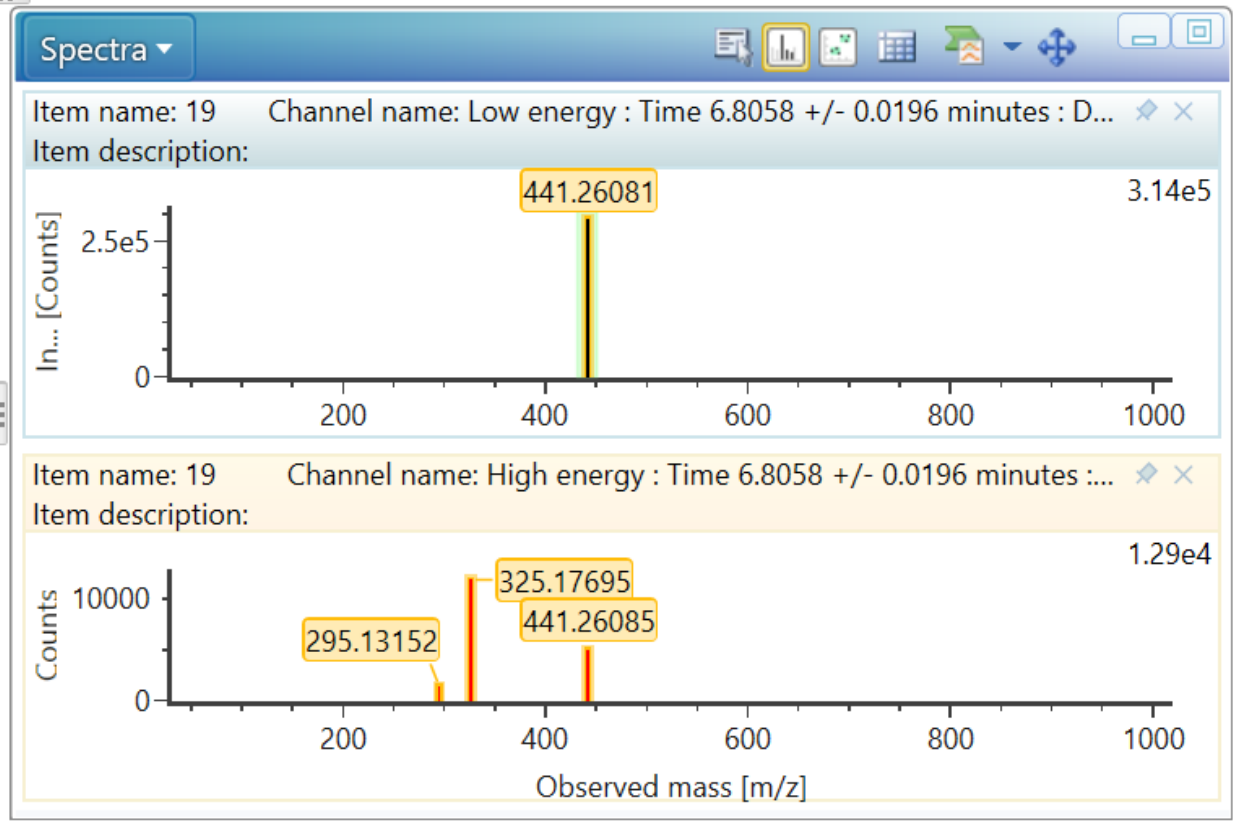
A paradigmatic case for the food supplement scenario

Drug-like supplement
Health Claim
Regulation for CIT

Righetti et al. 2021

CASE STUDY 1 – Adulterants in Red Yeast Rice

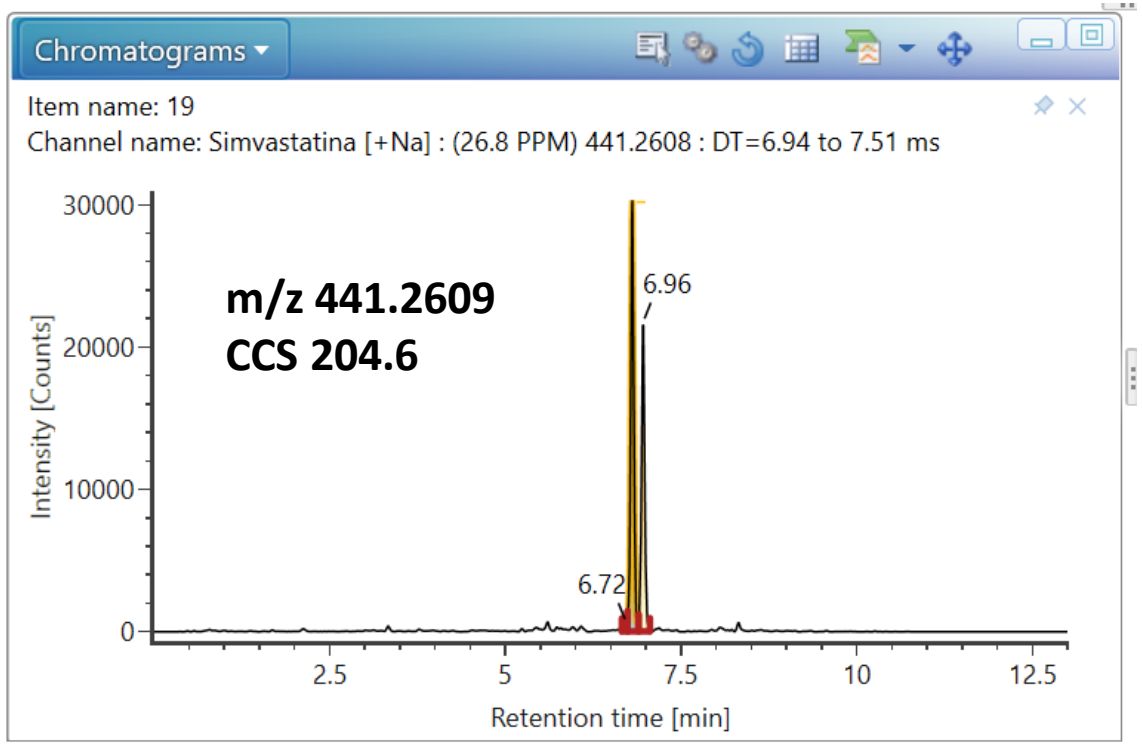


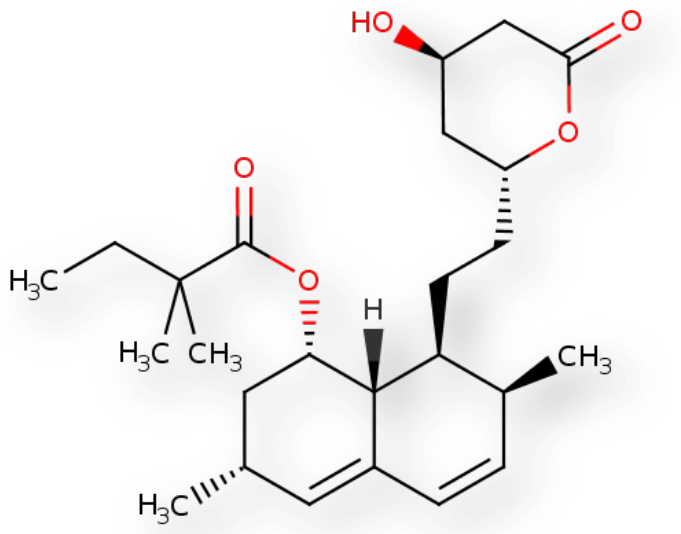


Fragmentation and RT consistent with a monacolins but not among the known natural compounds

However occurring at very low amount in the large majority of samples

Righetti et al. 2021



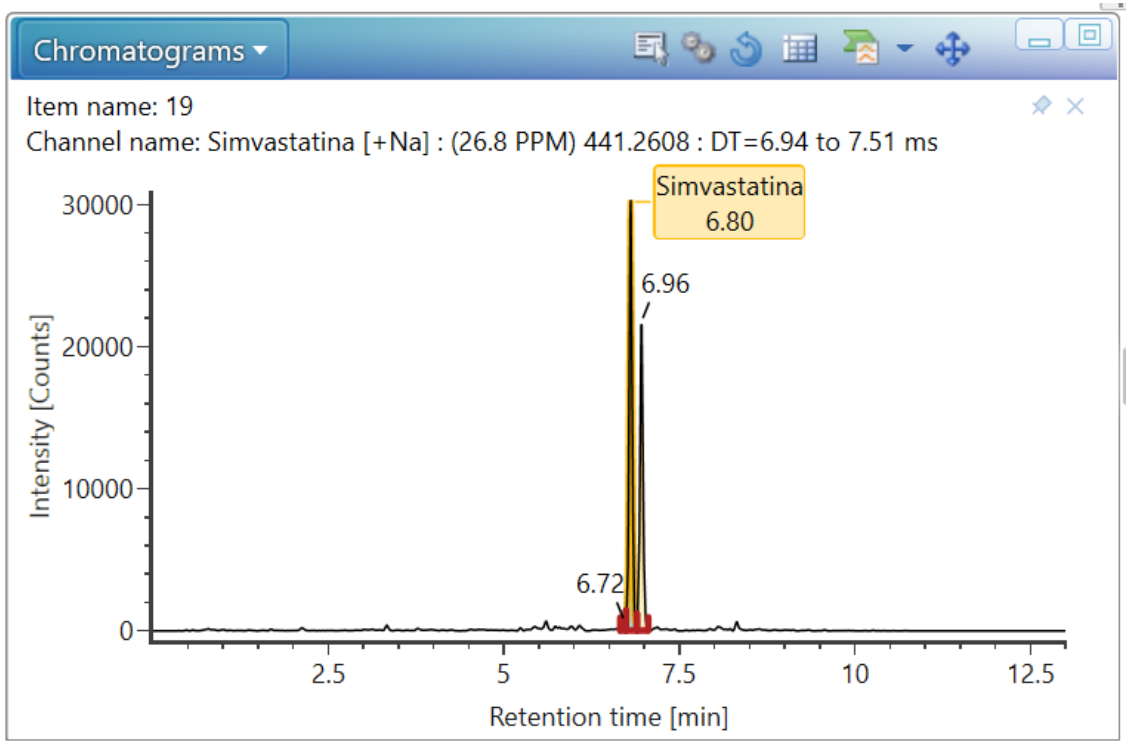


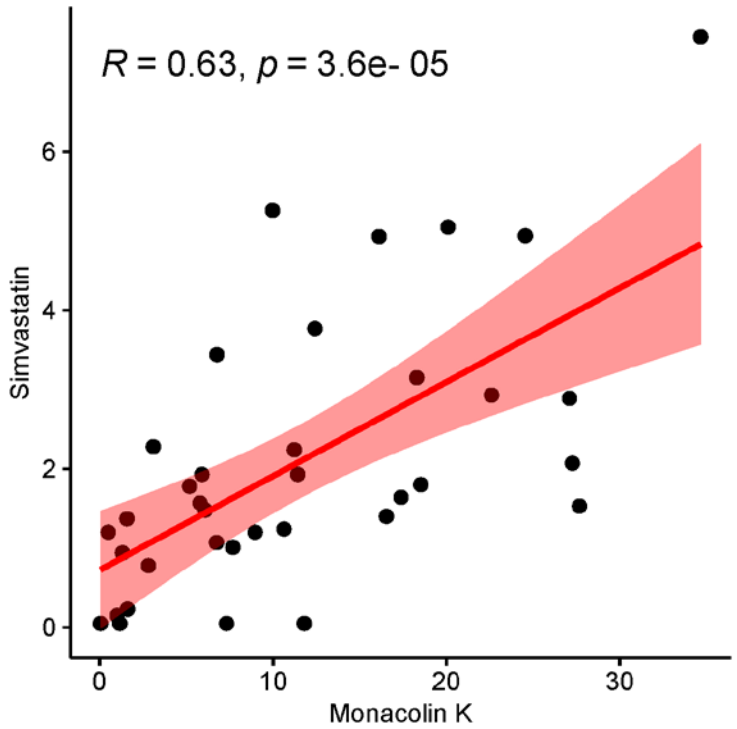
SIMVASTATIN

[M+Na]+
ΔCCS – 1.68%
ppm – 0.2

T3DB

A query based on MS and MS/MS spectra plus comparison of observed and predicted CCS





A significant correlation with MK (lovastatin) content

Since MKA is the main form in RYR, this might be due to addition of synthetic lovastatin

Take Home Message… and what we still miss

CASE STUDY 1
IMS can help in well-known adulterant identification

CASE STUDY 2
Machine-learning driven CCS prediction and online sources can support compound annotation

1. Lack of DB collection, curation and harmonization

2. Lack in Interoperability (often vendor-based)

3. Lack of easy-to-handle tools for routinary analysis

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CASE STUDY 3 – Species substitution in rhodiola supplements

Rhodiola Rosea Extract

Rhodiola is an herb that grows in the cold, mountainous regions of Europe and Asia.

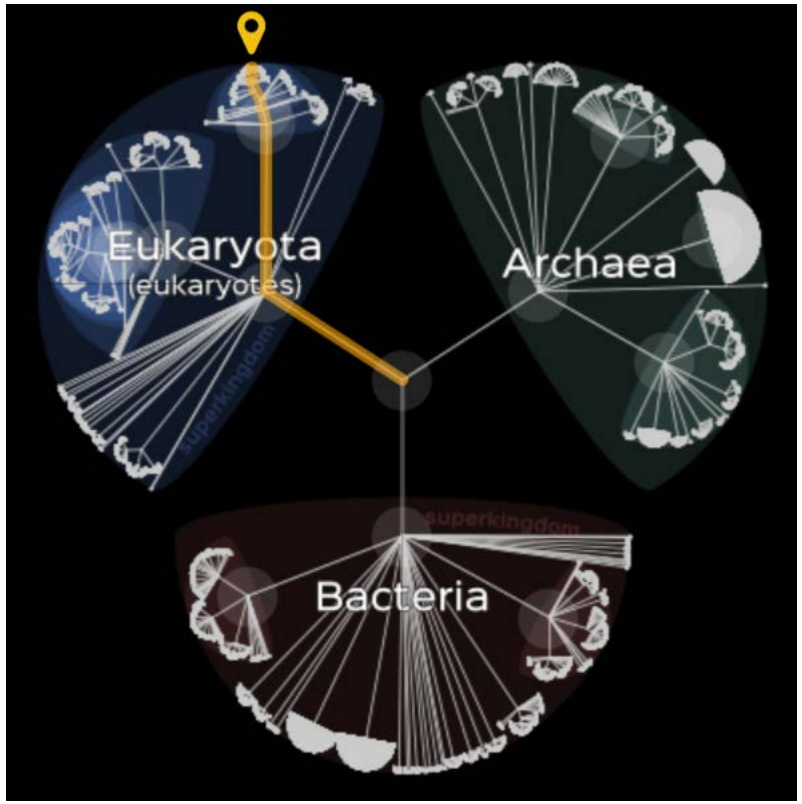
Its roots are considered **adaptogens**, meaning it may help strengthen the response to physical and psychological stressors.

Rhodiola rosea is included in the herb medicine list.

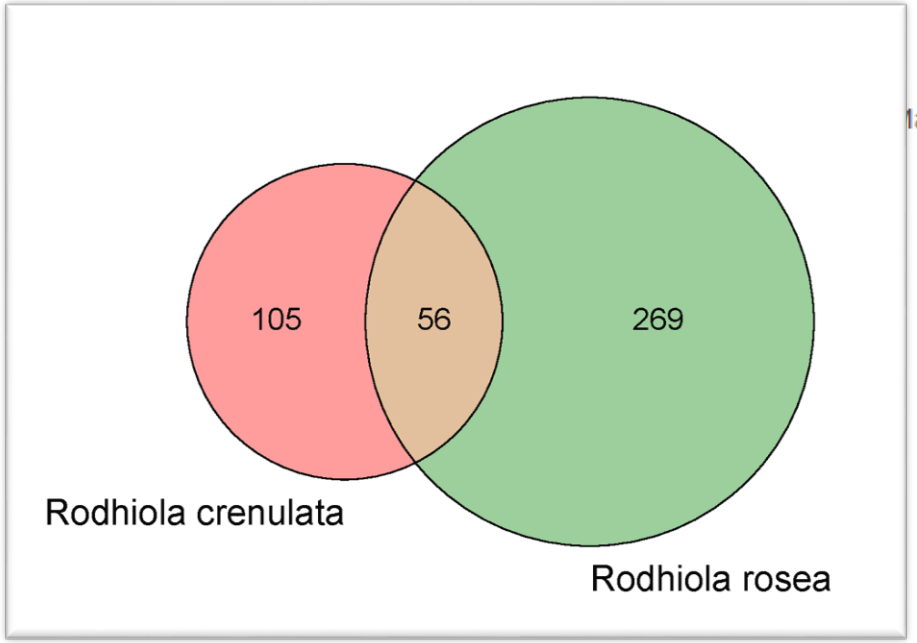
Its close-relative species *R. crenulata* is not

R. crenulata is very common in China and often used for extracts.

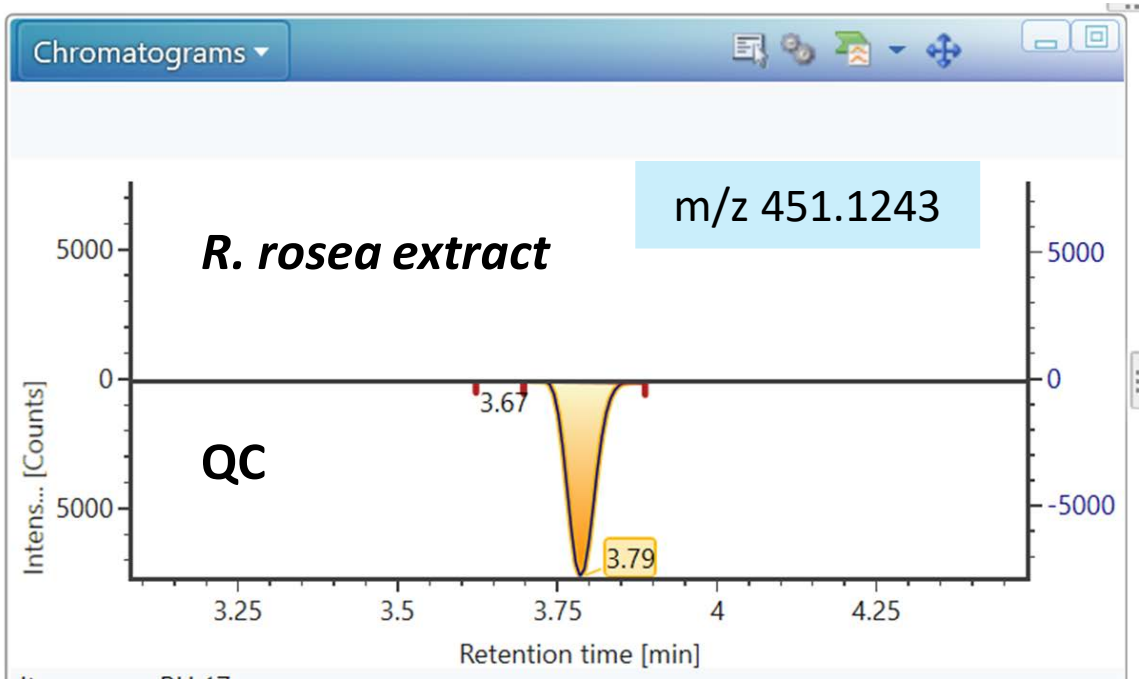
NCBI Taxonomy Browser

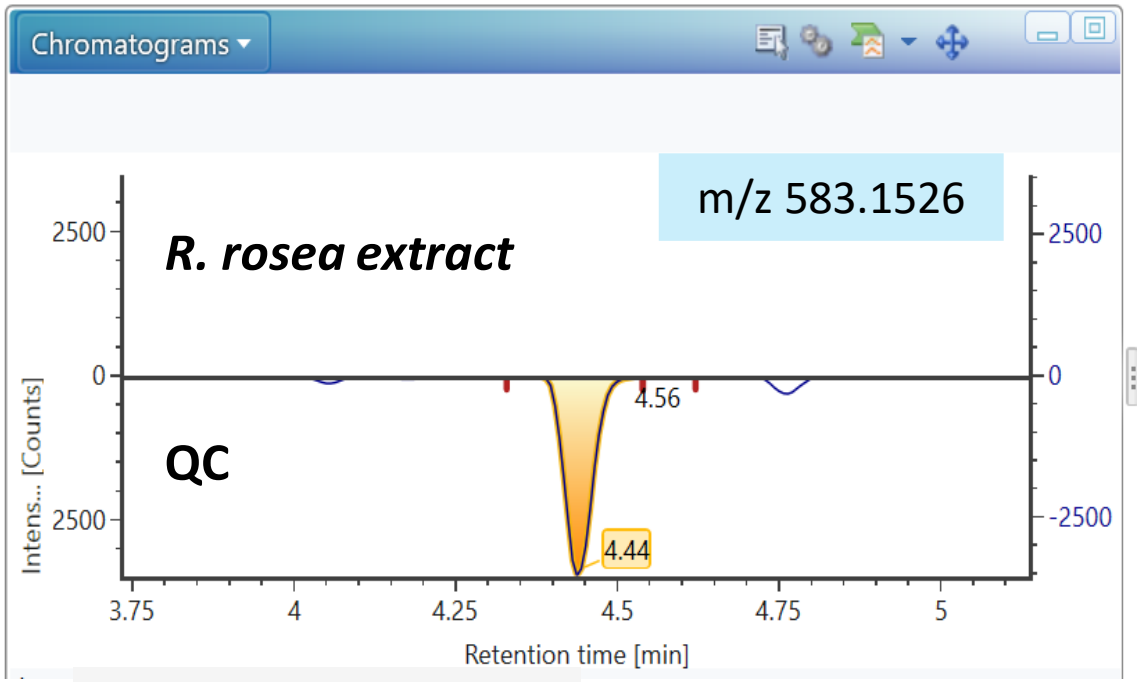


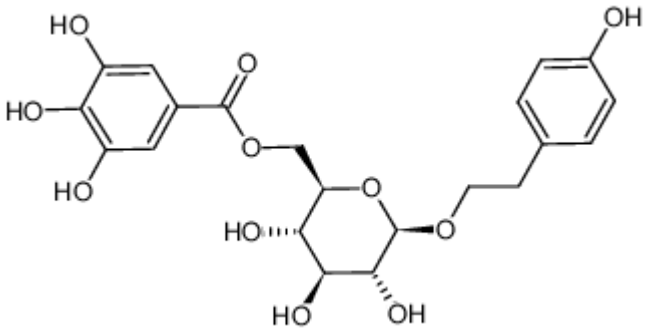
- LOTUS Tree ? ↗ 215,928
  - Biological Tree 194,272
    - Eukaryota 176,766
      - Archaeplastida 133,703
        - Streptophyta 131,578
          - Cupressales 3,092
            - Cupressaceae 2,110
              - Juniperus 844
                - Juniperus communis 197
  - lagnoliopsida 124,388
    - Lamiales 13,811
      - Plantaginaceae 1,411
        - Bacopa 118
          - Bacopa monnieri 115
      - Saxifragales 2,220
        - Crassulaceae 889
          - Rhodiola 410
            - Rhodiola crenulata 86
            - Rhodiola rosea 293
            - Rhodiola sachalinensis 108



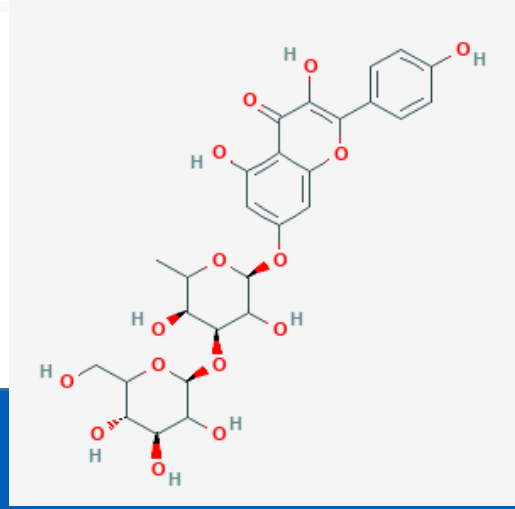
Comparison of rhodiola supplements with an authentic *R. rosea* extract



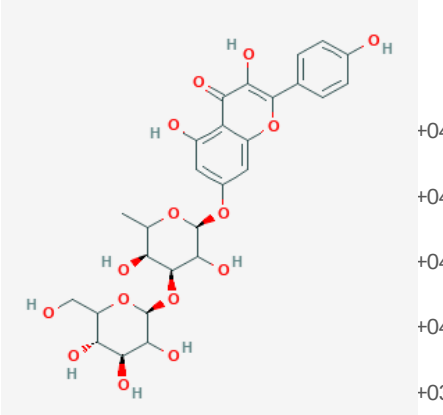


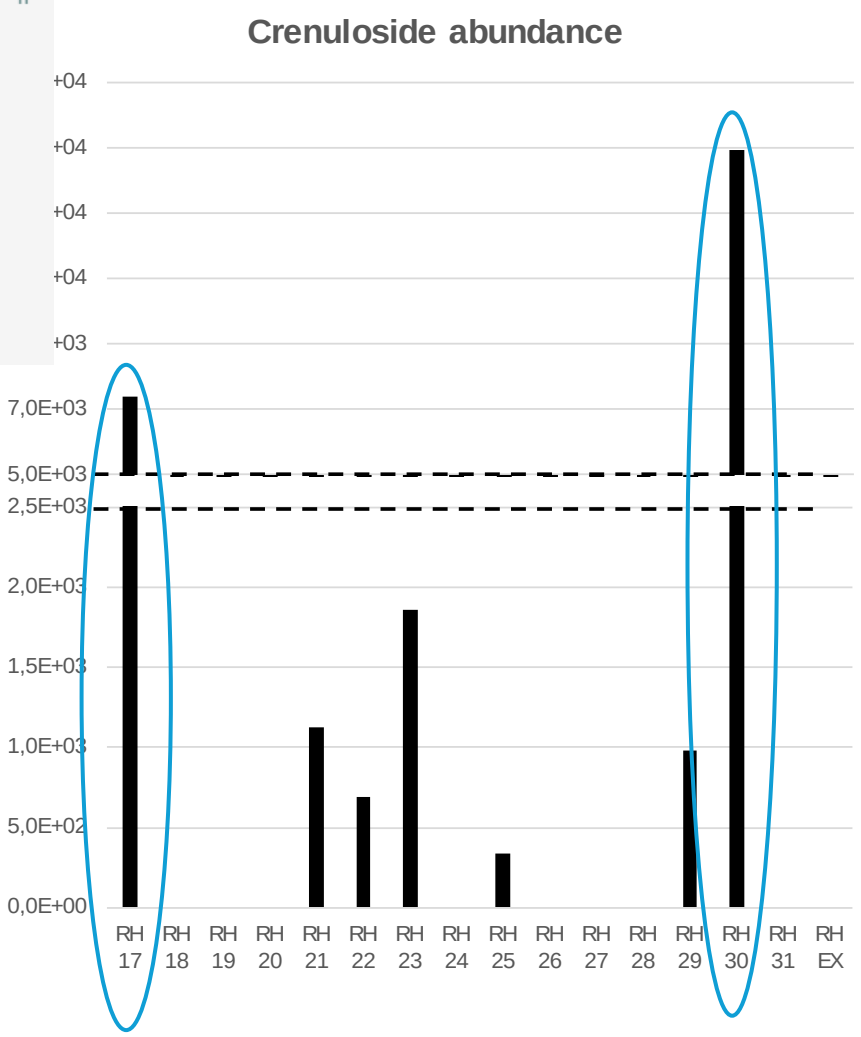


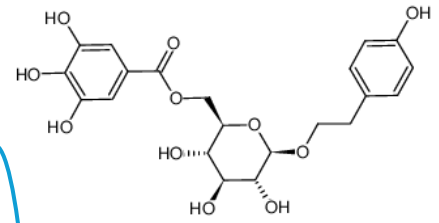
6-O-galloyl-salidroside

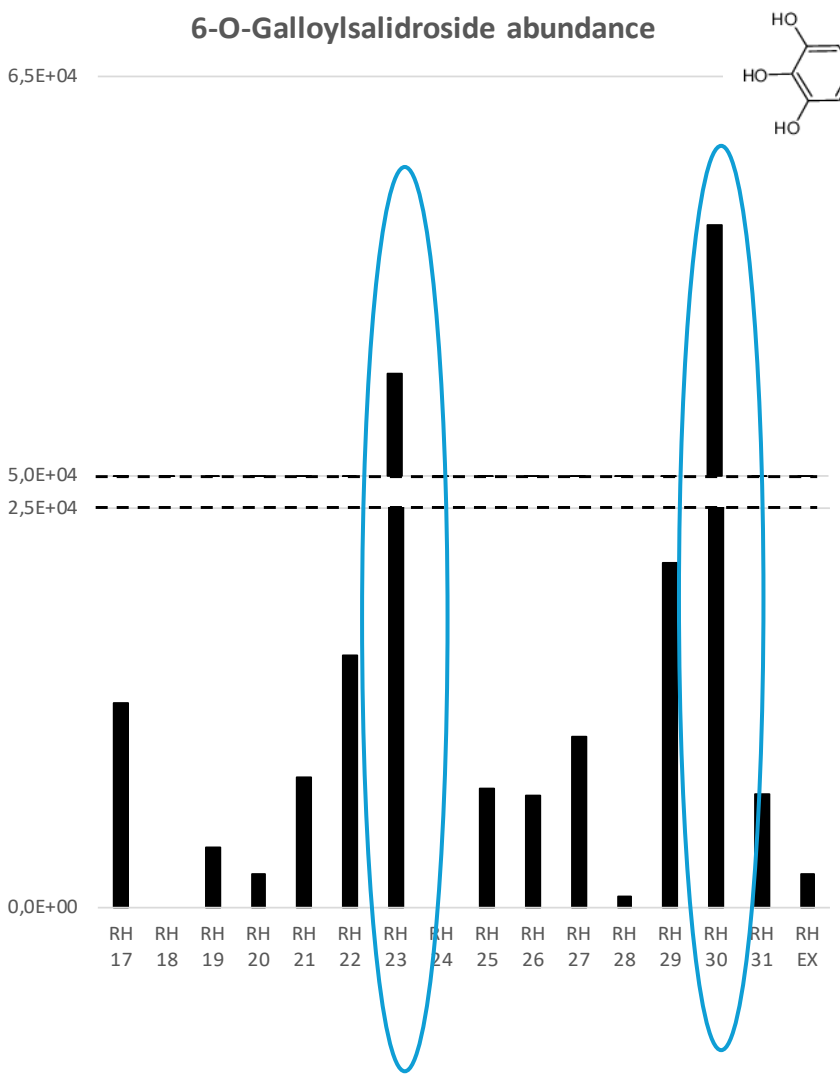


Crenuloside




Crenuloside abundance
+04
+04
+04
+04
+03
7,0E+03
5,0E+03
2,5E+03
2,0E+03
1,5E+03
1,0E+03
5,0E+02
0,0E+00
RH 17
RH 18
RH 19
RH 20
RH 21
RH 22
RH 23
RH 24
RH 25
RH 26
RH 27
RH 28
RH 29
RH 30
RH 31
RH EX




6-O-Galloylsalidroside abundance
6,5E+04
5,0E+04
2,5E+04
0,0E+00
RH 17
RH 18
RH 19
RH 20
RH 21
RH 22
RH 23
RH 24
RH 25
RH 26
RH 27
RH 28
RH 29
RH 30
RH 31
RH EX