

Quantitative analysis of plant phenolics by LC-MS/MS, and PhenolicsDB: a publicly available high-resolution MS/MS spectral library

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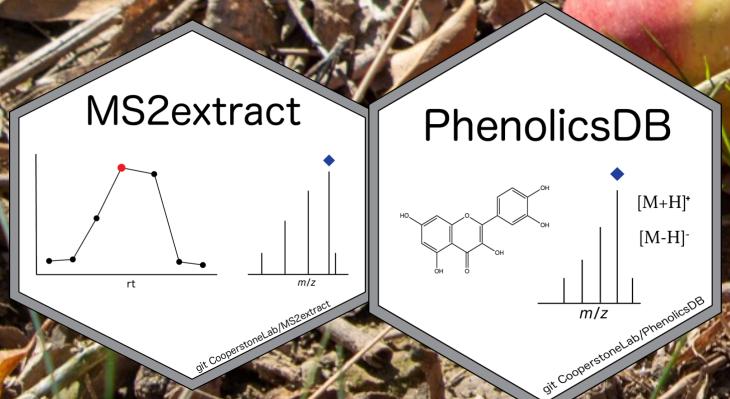
DanielQuiroz97



cristianquirozd



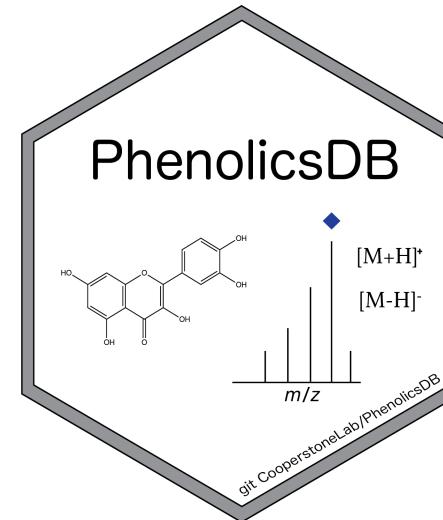
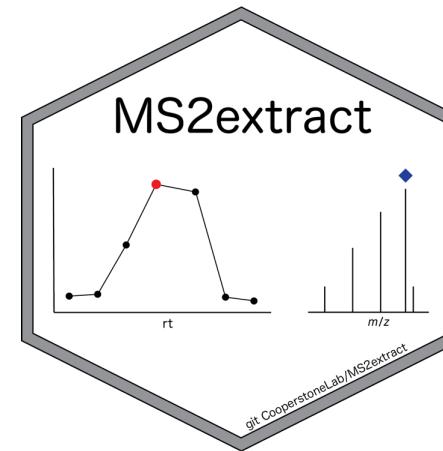
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Outline

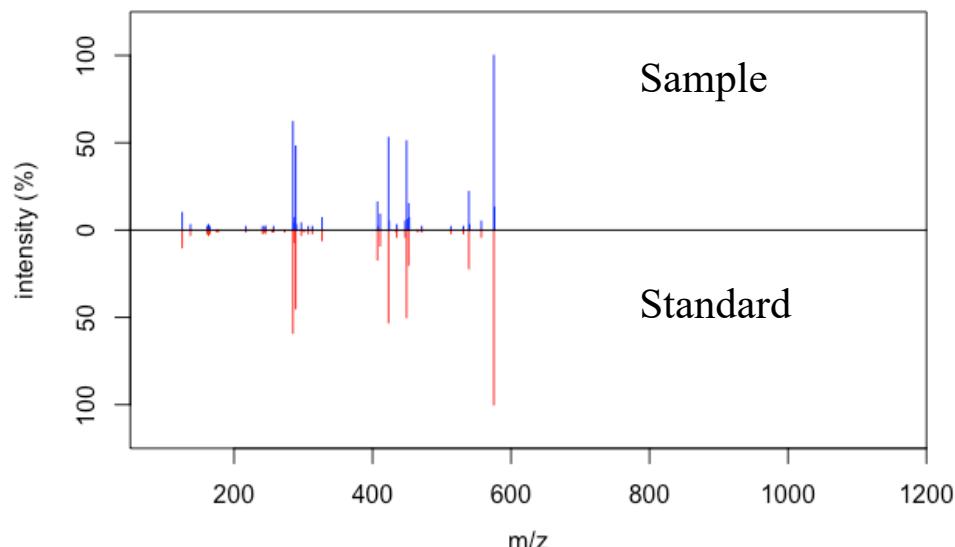
- Inspiration to develop MS2extract
- PhenolicsDB: HR MS/MS library
- Phenolics quantification method – Extraction
- Phenolics quantification method – LC-QqQ



Metabolite Identification



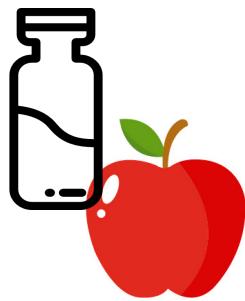
Level	Criteria
Level 1 (gold standard)	Comparison with authentic standards
Level 2	Putatively annotated with public/commercial spectral libraries
Level 3	Compound class
Level 4	Unknown compounds - features



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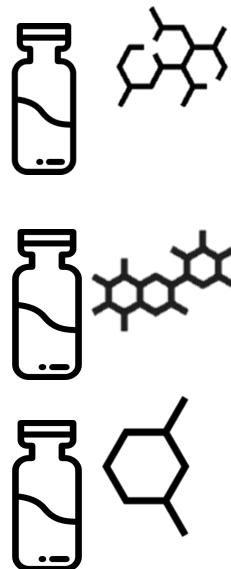
Manual Metabolite ID is labor intensive

Sample

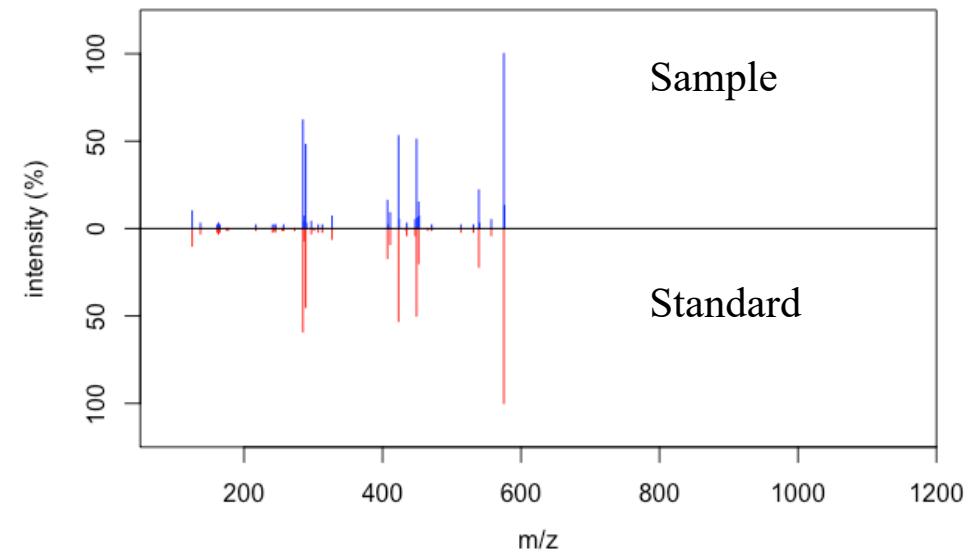


+

Standards



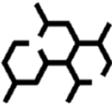
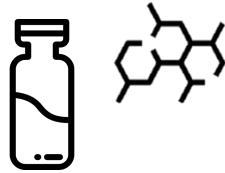
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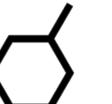
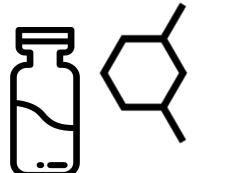
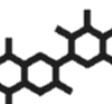
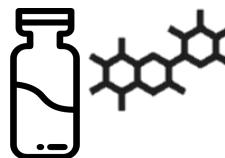
How many standards are too many?

There is nothing like having too many standards, but...



Standards classes

- Phenols
- Flavonoids
- Dihydrochalcones
- Organic acids
- Sugars



n = 73

Collision energy

- CE = 20 eV
- CE = 40 eV

n = 2

Ionization mode

- Positive
- Negative

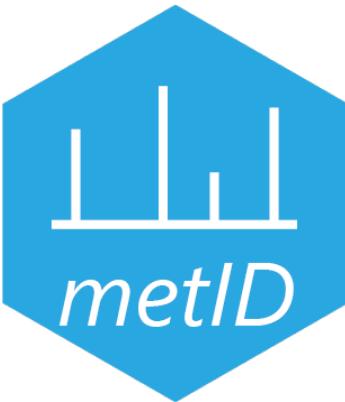
n = 2

$$\text{Number of MS/MS spectra files} = 73 * 2 * 2 = 292$$



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Open-source software alternatives

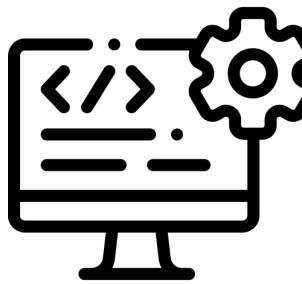
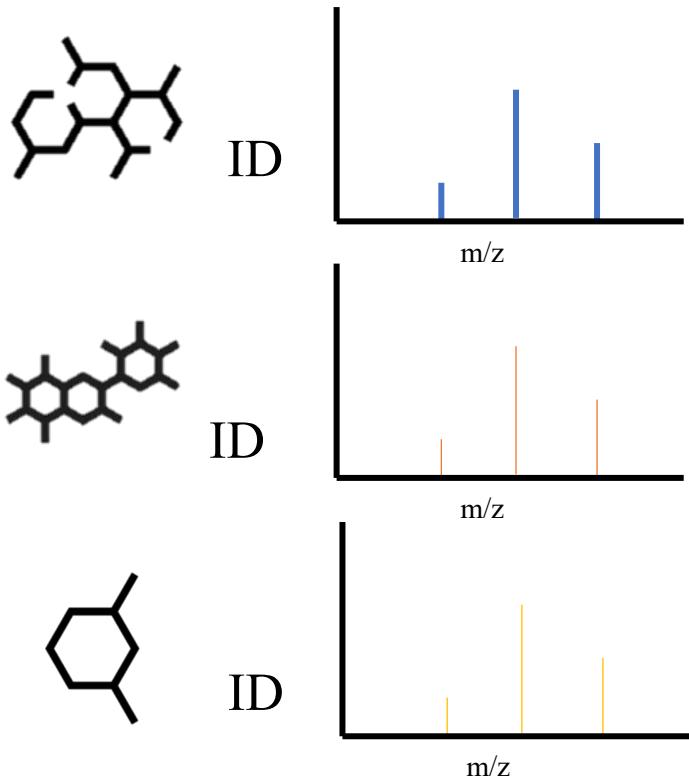


The MS/MS spectra of the most intense MS^2 scan is exported



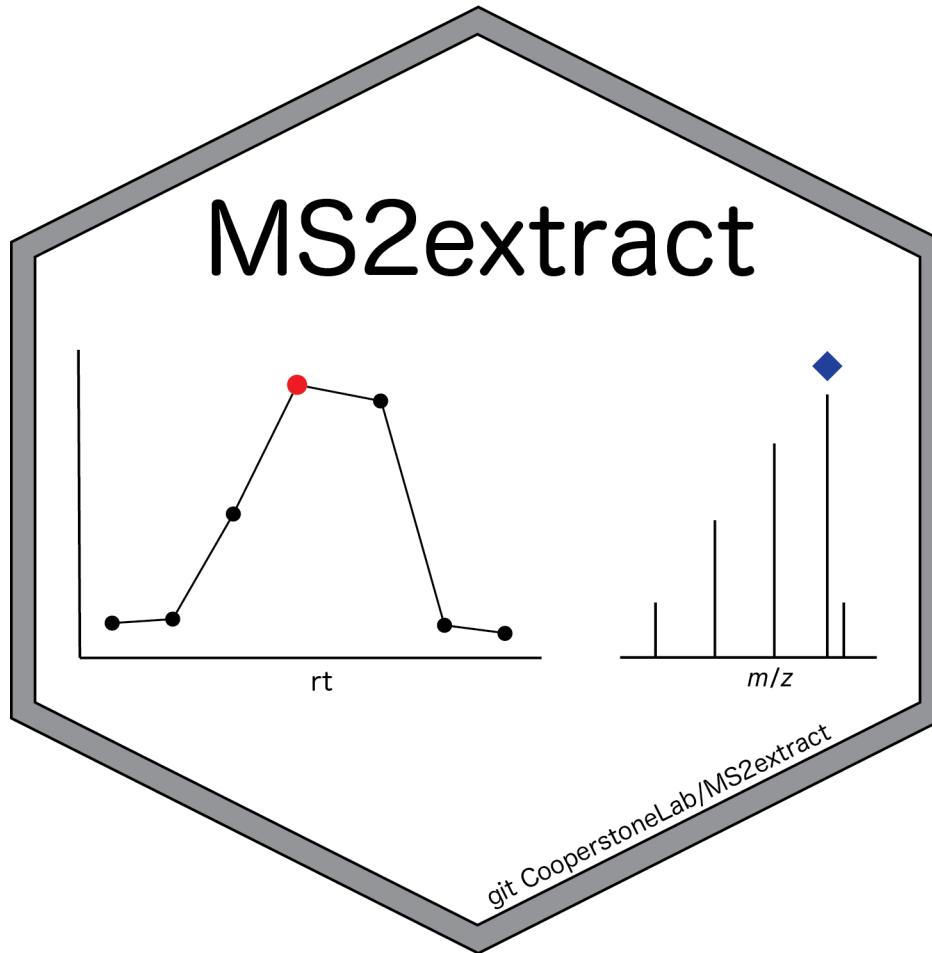
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An open-source tool was missing

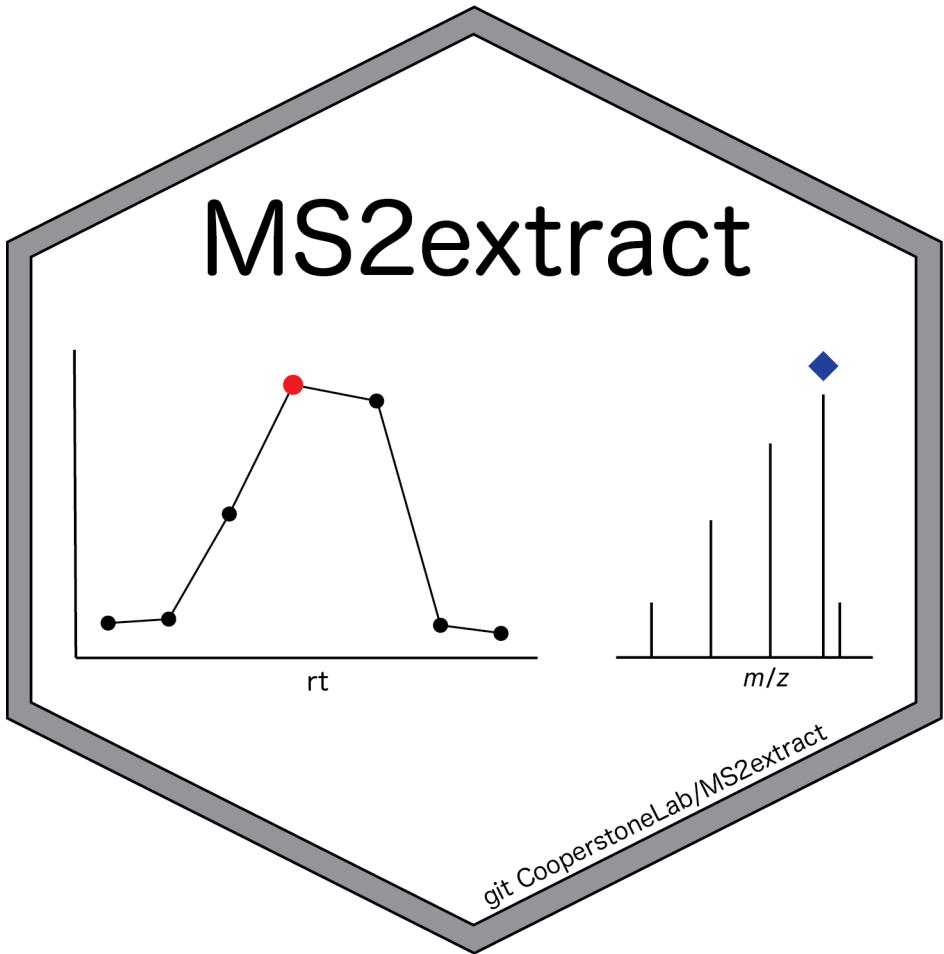


Software





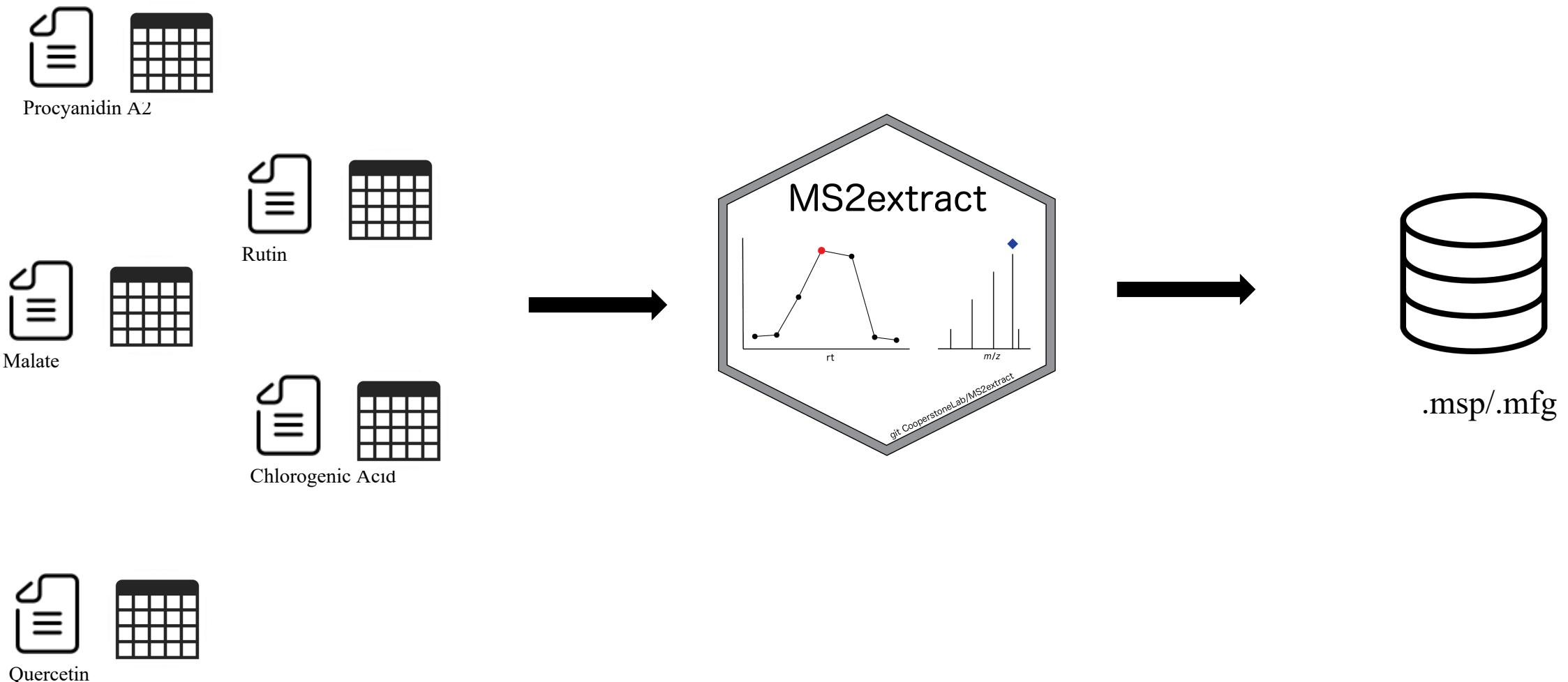
<https://github.com/CooperstoneLab/MS2extract>



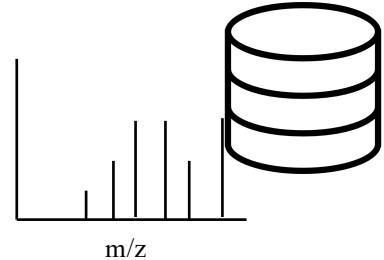
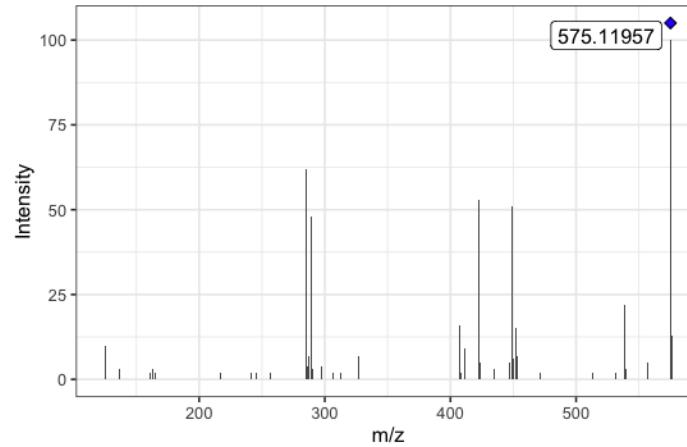
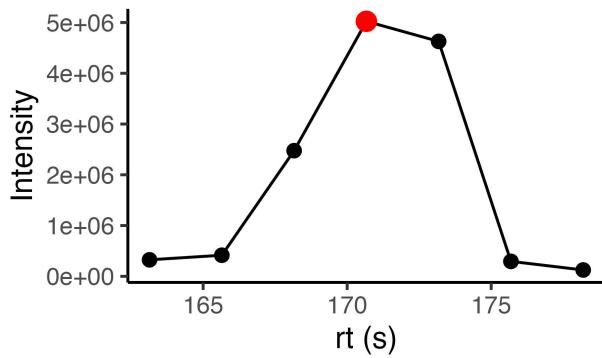
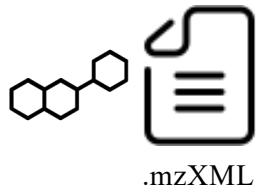
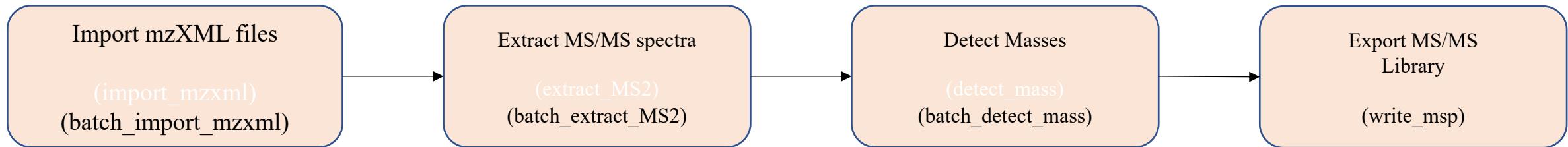
MS2extract is an R package that offers the option to create in-house MS/MS compound libraries

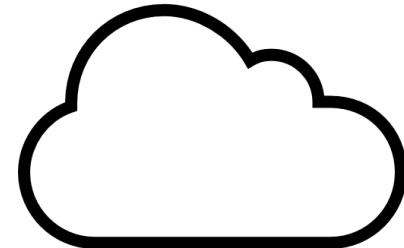
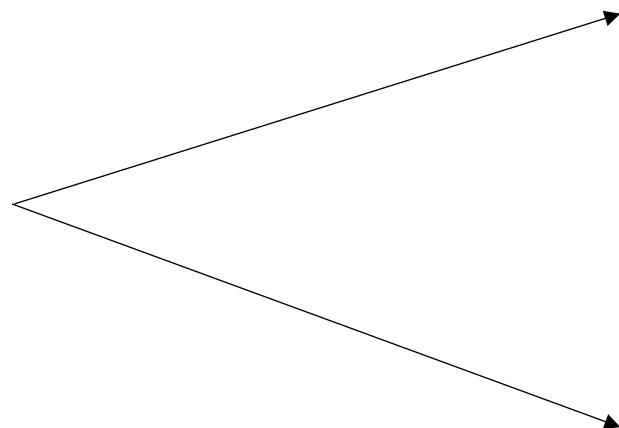
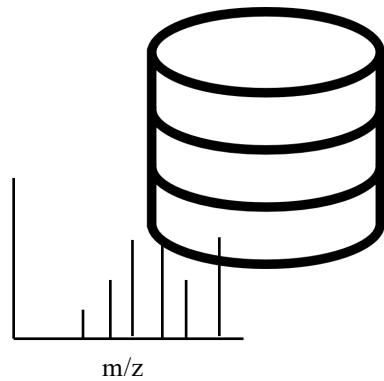
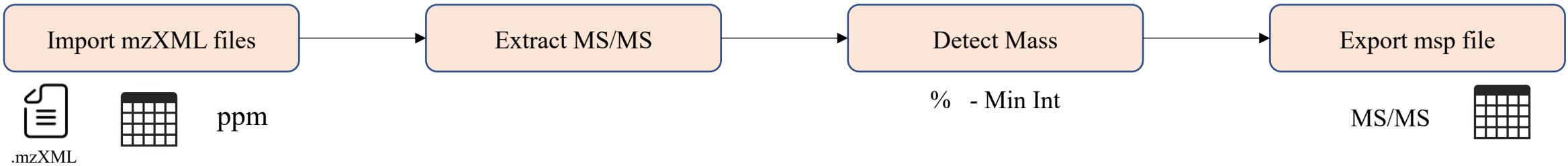
<https://github.com/CooperstoneLab/MS2extract>

MS2extract enables the automatization of MS/MS library creation



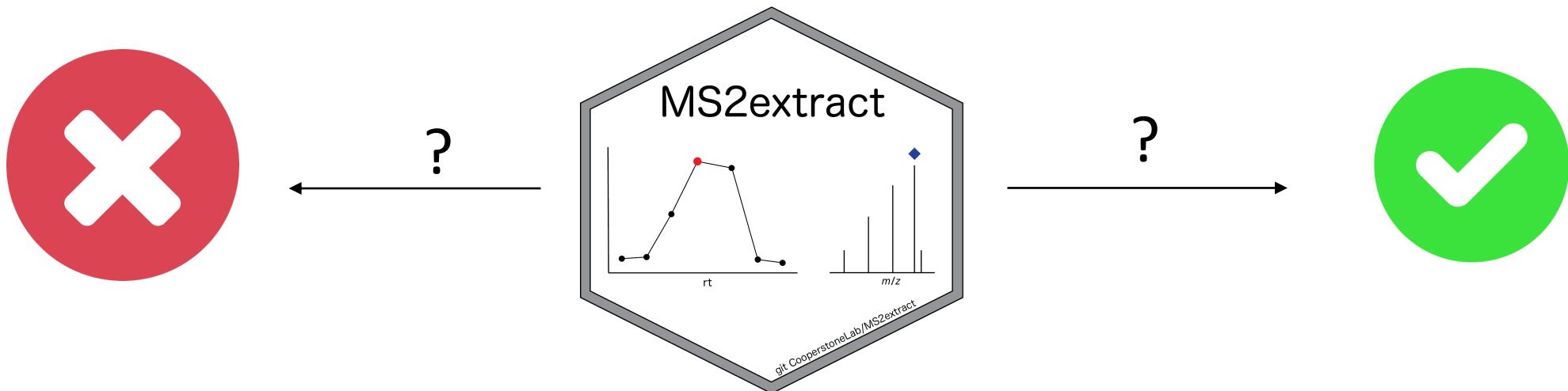
MS2extract Pipeline





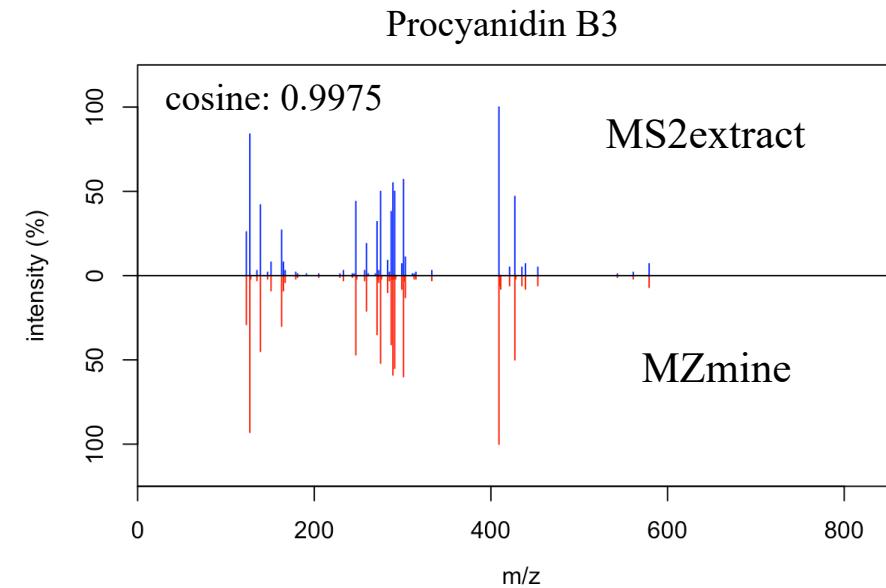
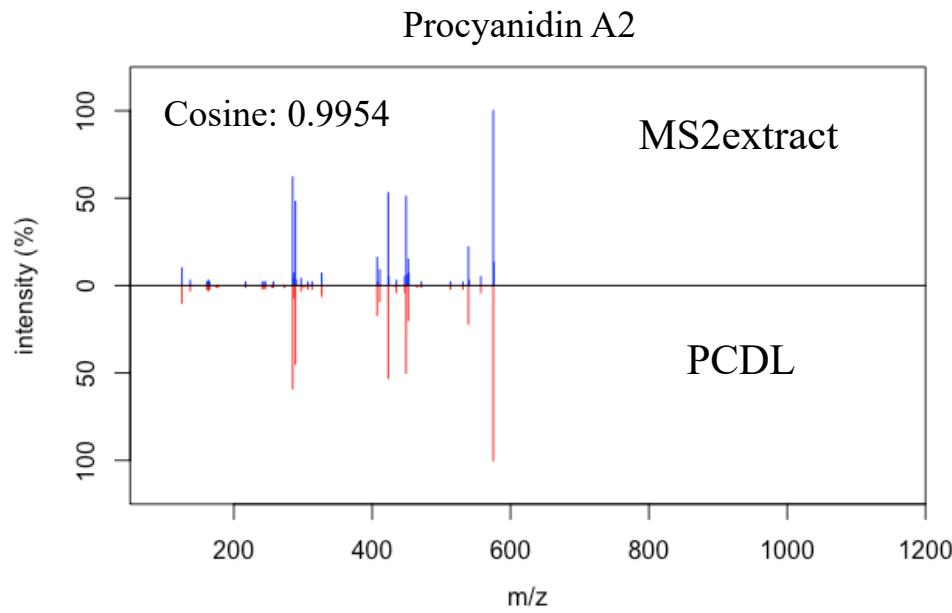
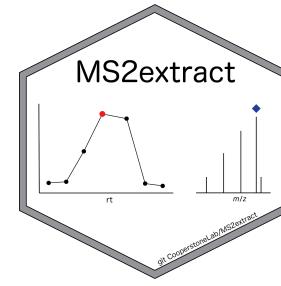
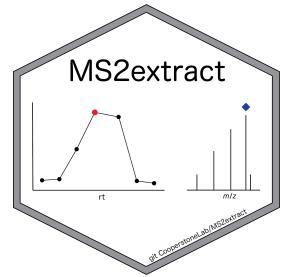
- GNPS (compatible)
- MASSIVE
- MASSBANK

How do we know MS2extract makes sense?



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MS2extract compared with known software as a positive control



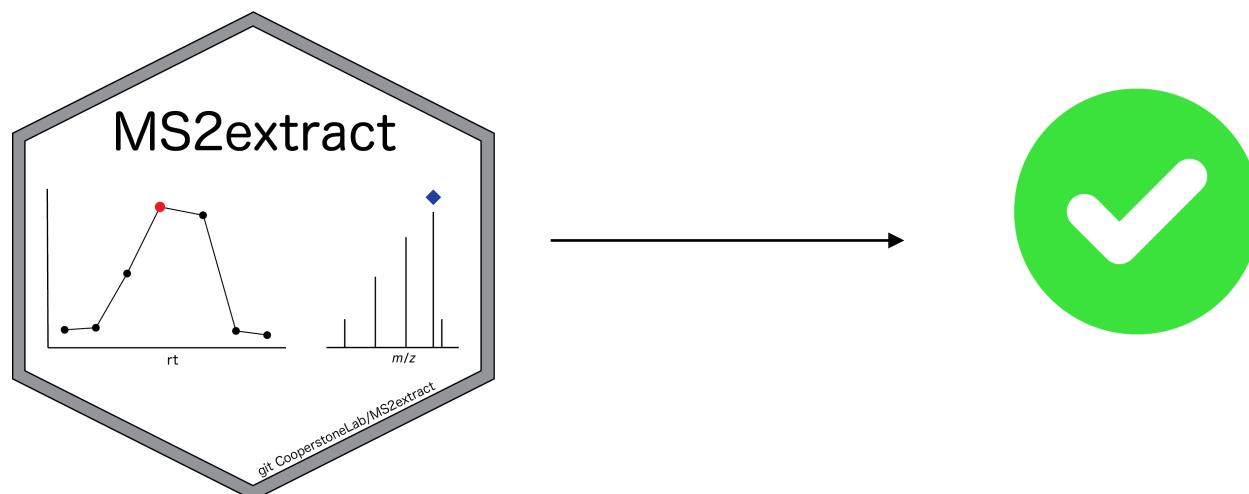
...

MS2extract cosine scores > 0.995



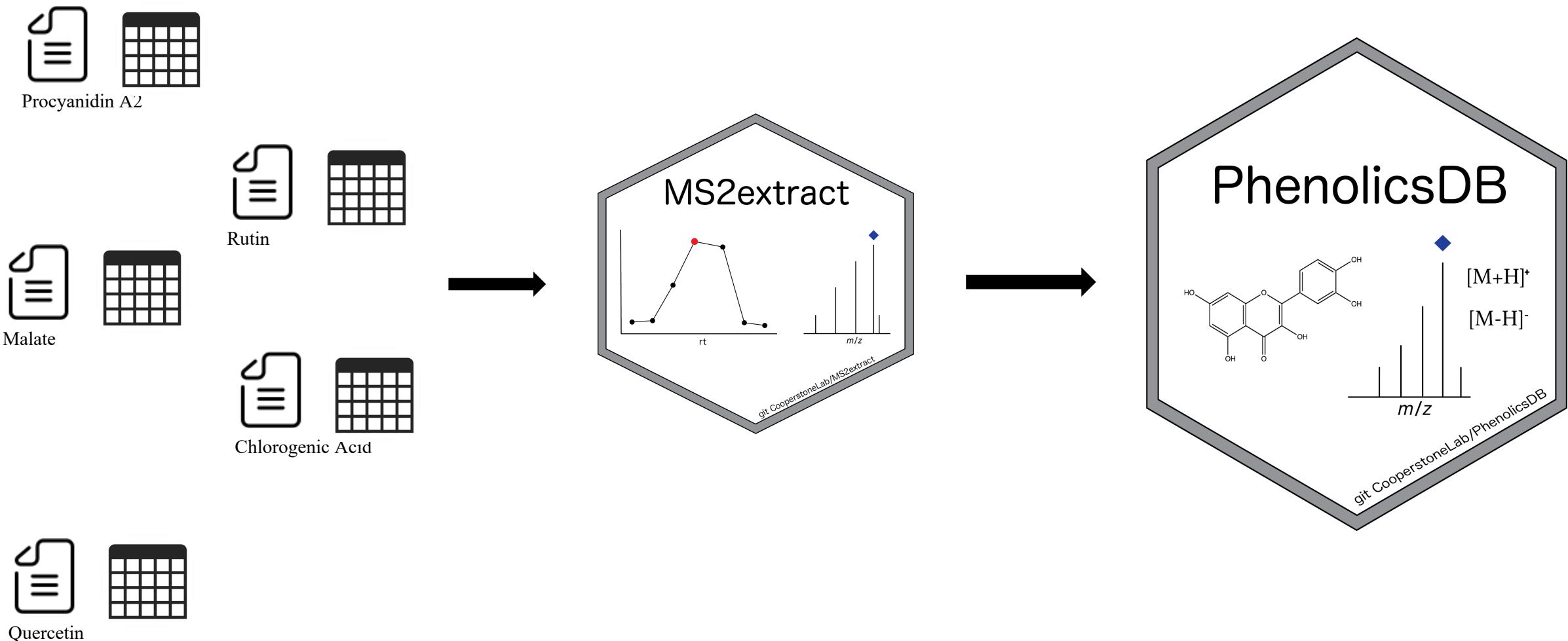
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MS2extract does make sense

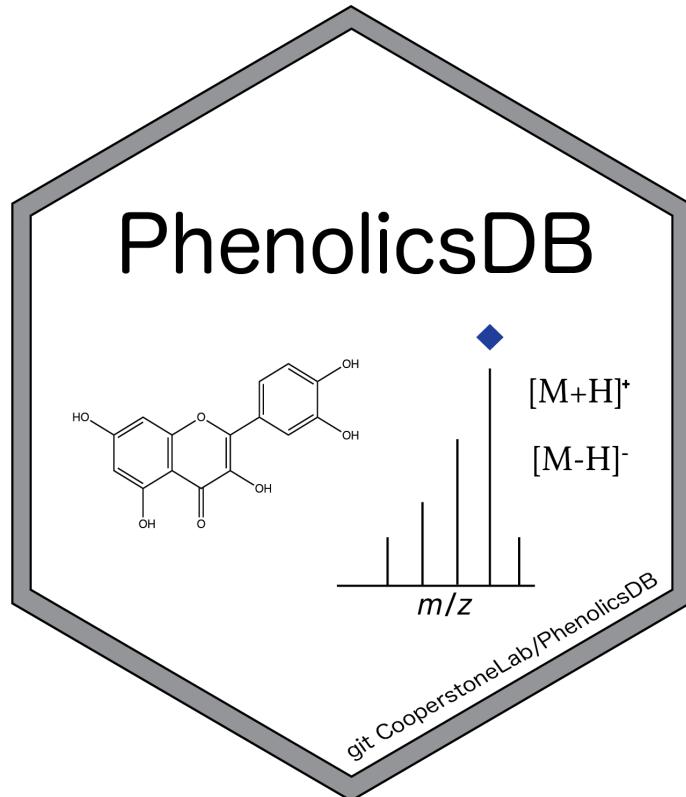


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MS2extract enables the automatization of MS/MS library creation



PhenolicsDB: a High-Resolution MS/MS library

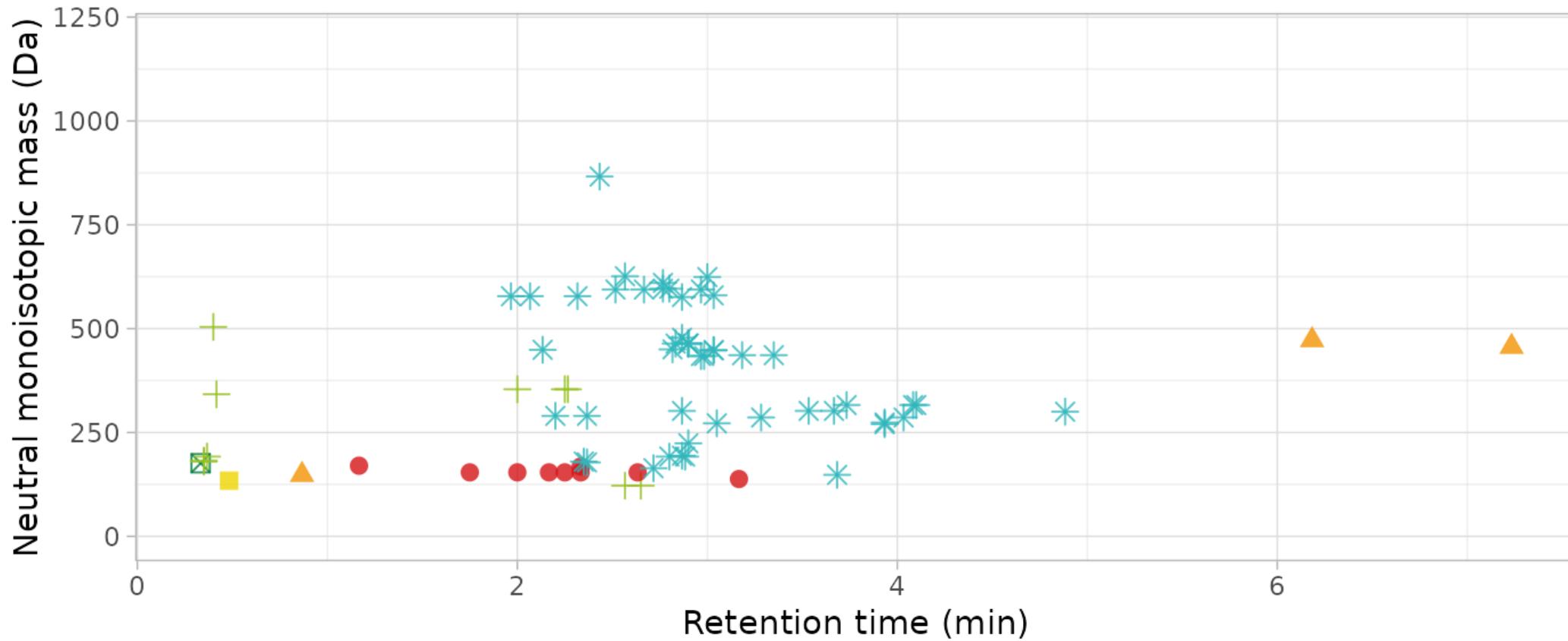


- 73 Analytical standards (mostly phenolics)
- 320 reference MS/MS
- Retention time information
- Positive and Negative polarity
- 20, 40, 60, and 80 CE (CID)
- Available formats (NIST .msp and GNPS .mgf)



Distribution of the metabolites included in PhenolicsDB

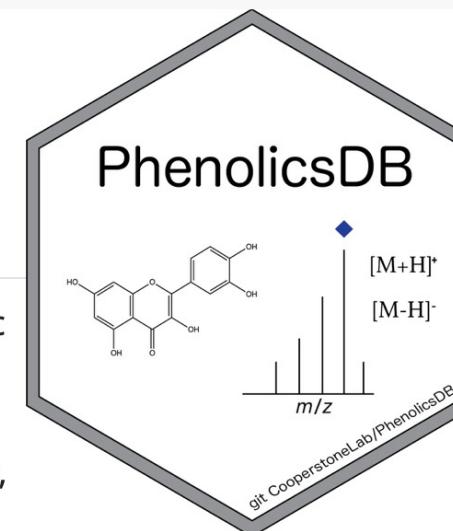
Metabolites are colored based on Superclass information from ClassyFire



Superclass_CF	● Benzenoids	✚ Organic oxygen compounds
	▲ Lipids and lipid-like molecules	▣ Organoheterocyclic compounds
	■ Organic acids and derivatives	* Phenylpropanoids and polyketides

PhenolicsDB

PhenolicsDB is a data package that contains raw MS/MS data of authentic analytical standards. PhenolicsDB raw data files and data frames are aimed to exemplify the use of [MS2extract](#) to create in-house metabolite MS/MS libraries. Between the list of metabolites we include phenolic acids, flavonoids, dihydrochalcones, carbohydrates, and others. Tandem mass spectrometry data was collected using a LC coupled to a Q-TOF mass analyzer.



Download PhenolicsDB

You can download PhenolicsDB in `.msp` and `.mgf` format in these links:

Format Positive polarity

NIST [Link](#)

`.msp`

GNPS [Link](#)

`.mgf`

Negative polarity

[Link](#)

[Link](#)

Links

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Citation

[Citing PhenolicsDB](#)

Developers

Cristian Quiroz-Moreno

Author, maintainer

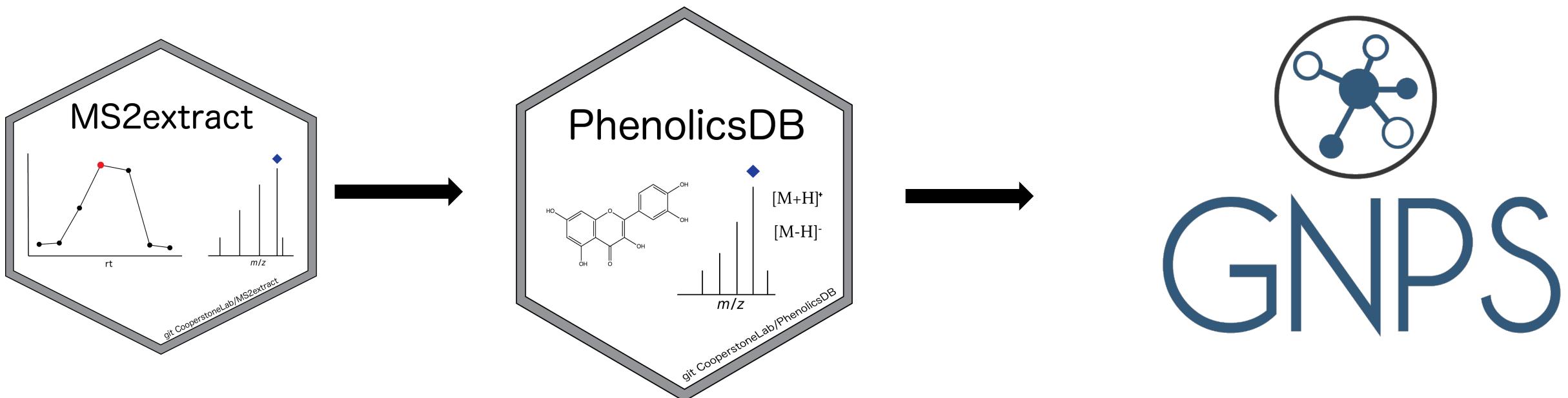
Jessica Cooperstone

Author

Dev status

DOI [10.5281/zenodo.10600772](#)

MS2extract enhances data reusability and sharing



CMMC-LIBRARY

GNPS

[Browse](#)

[Download](#)

[Download](#)

[Download](#)

PHENOLICSDB

GNPS

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[Download](#)

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CMMC-REFRAME-NEGATIVE-LIBRARY

GNPS

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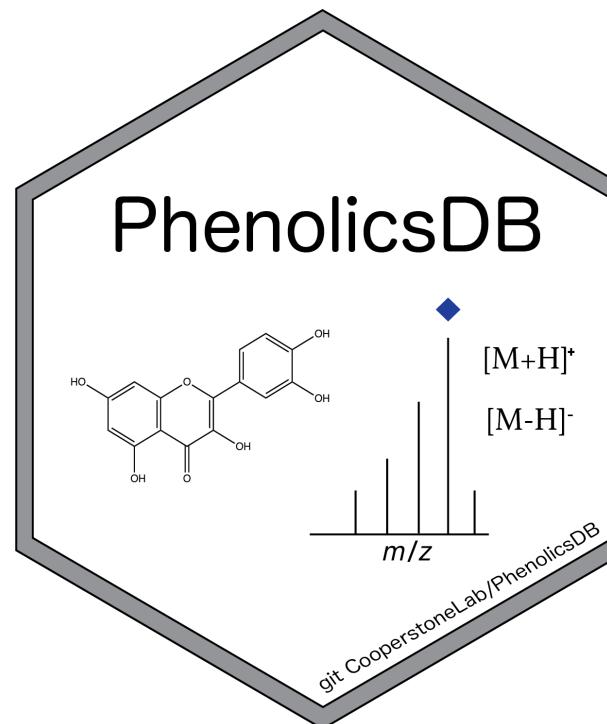
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<https://external.gnps2.org/gnpslibrary>

PhenolicsDB revealed the apple phenolics profile



15 Phenolics identified

- Malic acid
- Catechin
- (-)Epicatechin
- Chlorogenic acid
- Cryptochlorogenic acid
- Phloridzin
- Quercitrin
- Cyanidin 3-*O*-glucoside
- Quercetin-3-*O*- α -l-arabinopyranoside
- Quercetin 3-*O*-galactoside
- Quercetin 3-*O*-glucoside
- Procyanidin B1
- Procyanidin B2
- Procyanidin C1
- Kaempferol 3-*O*-rutinoside

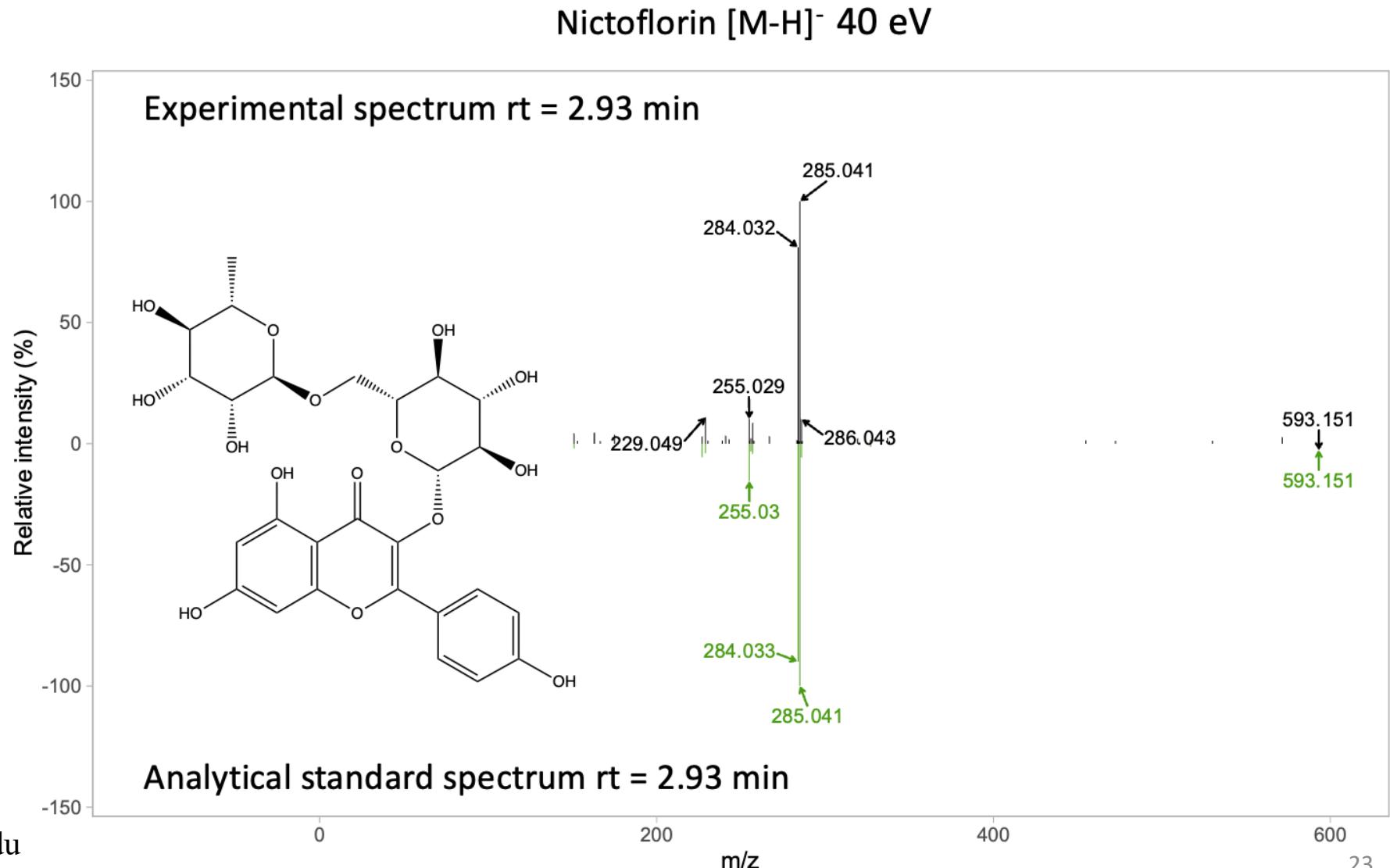
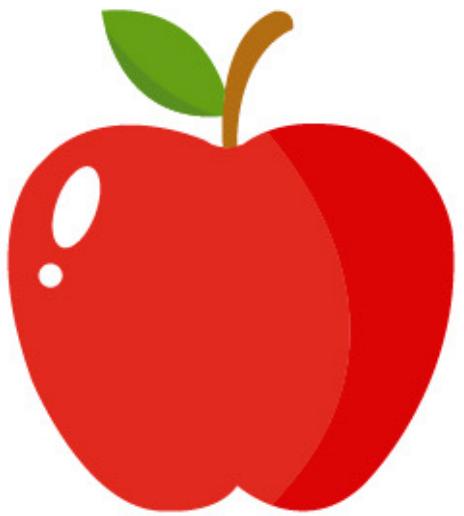
<https://cooperstonelab.github.io/PhenolicsDB/>



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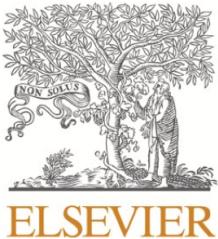
THE OHIO STATE UNIVERSITY COLLEGE OF FOOD, AGRICULTURAL, AND ENVIRONMENTAL SCIENCES

Case example: apple phenolics discovery



PhenolicsDB can be widely used

Microchemical Journal 199 (2024) 110058



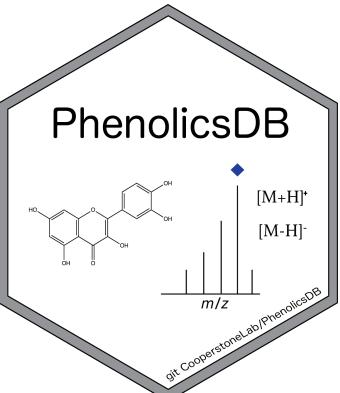
Contents lists available at [ScienceDirect](#)

Microchemical Journal

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Metabolite fingerprinting of *Urospatha sagittifolia* (Araceae) tubers at different growth stages by multi-platform metabolomics and molecular networking



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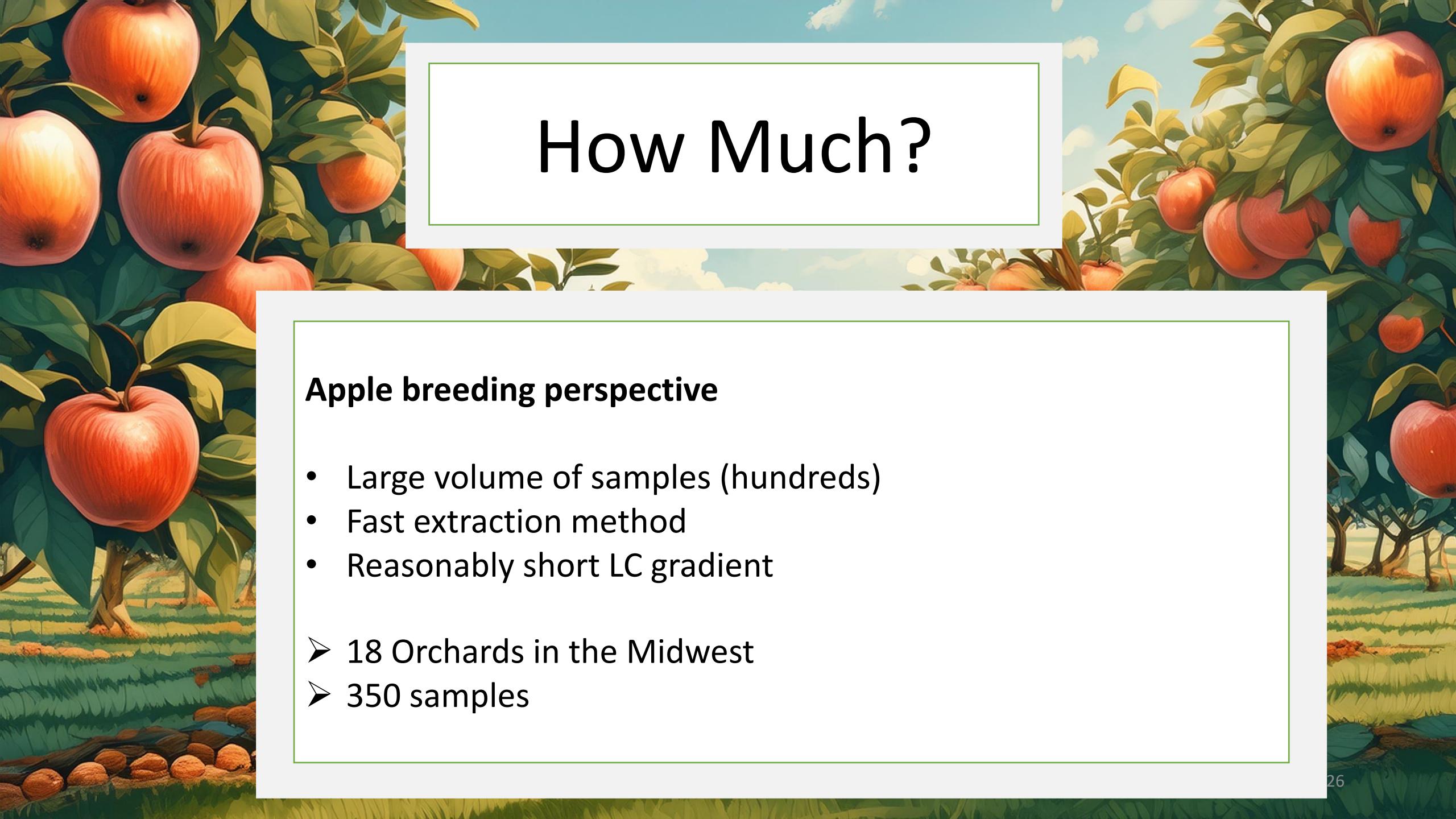
Untargeted Characterization and Biological Activity of Amazonian Aqueous Stem Bark Extracts by Liquid and Gas Chromatography- Mass Spectrometry



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How Much?

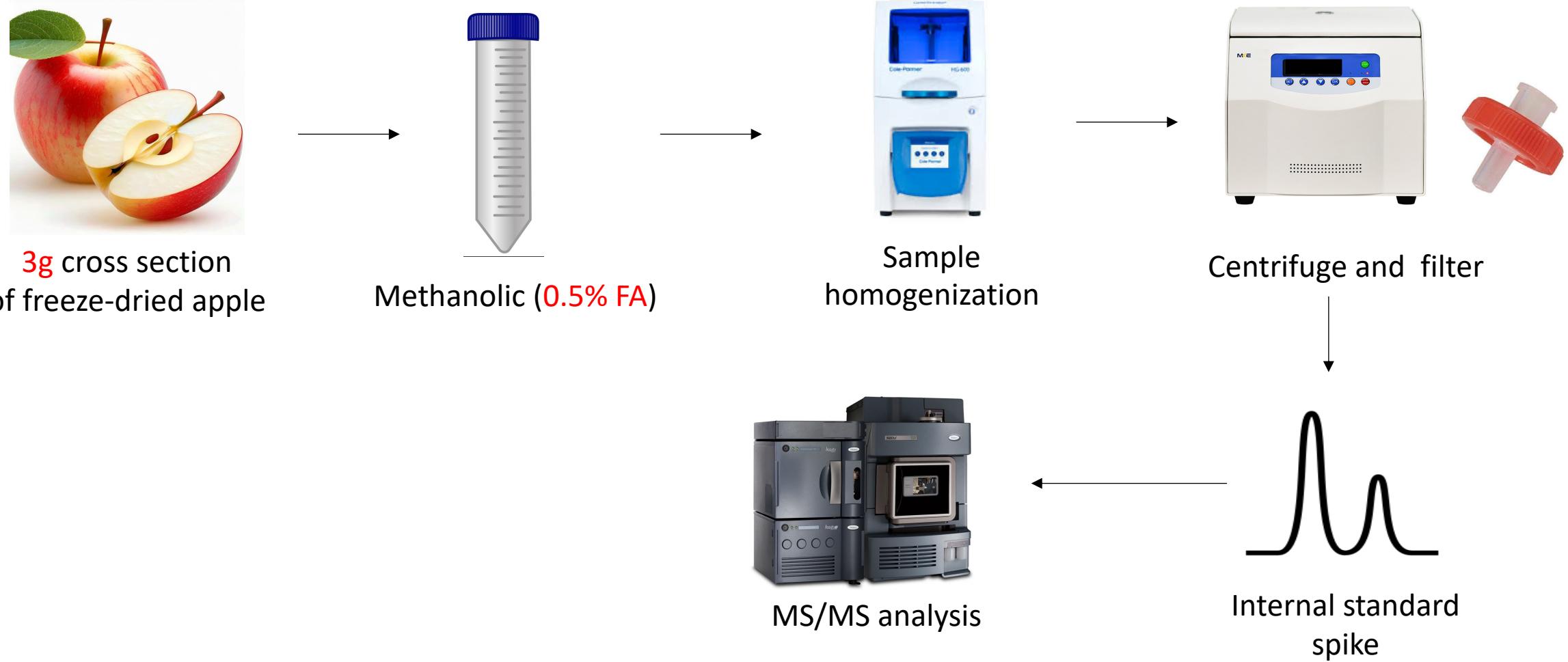


How Much?

Apple breeding perspective

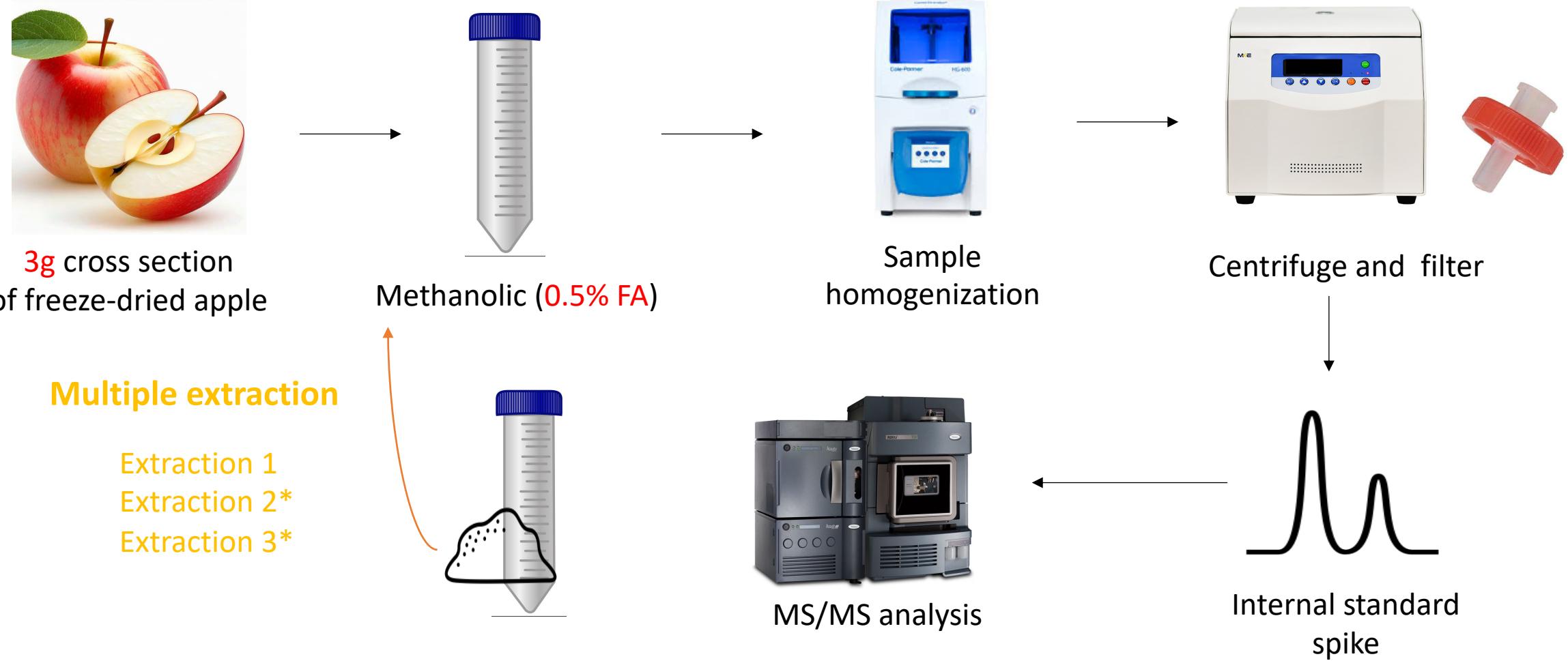
- Large volume of samples (hundreds)
 - Fast extraction method
 - Reasonably short LC gradient
- 18 Orchards in the Midwest
- 350 samples

Phenolics quantification method - Extraction



Is the extraction efficiency 100%?

Phenolics quantification method - Extraction

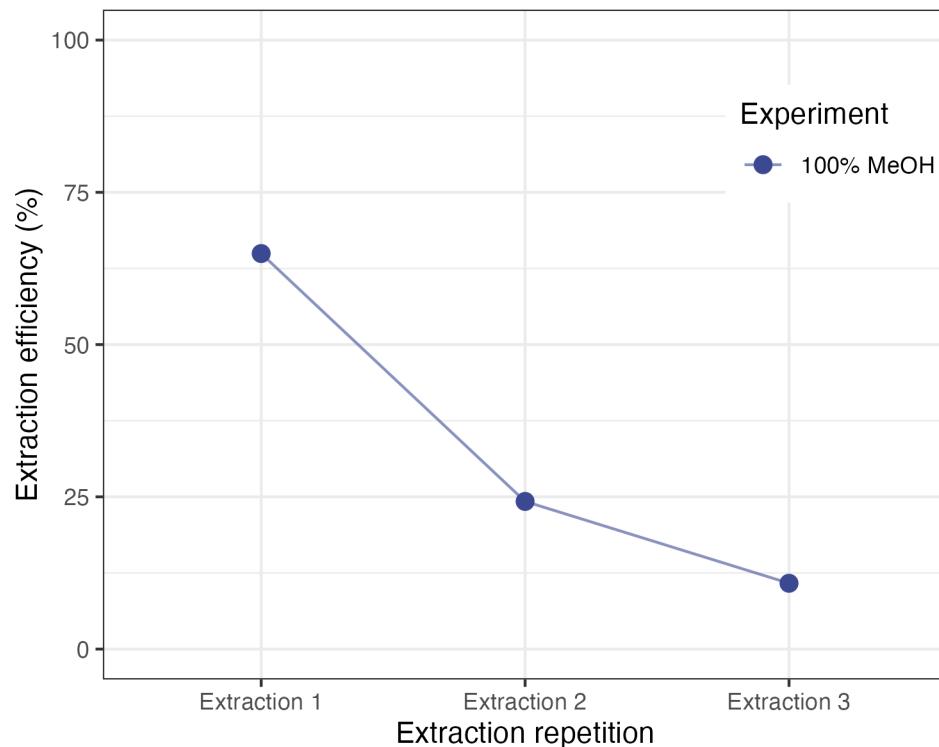


Is the extraction efficiency 100%?

Analyte: Chlorogenic acid

Apple accession: DW90

Chlorogenic content: Low



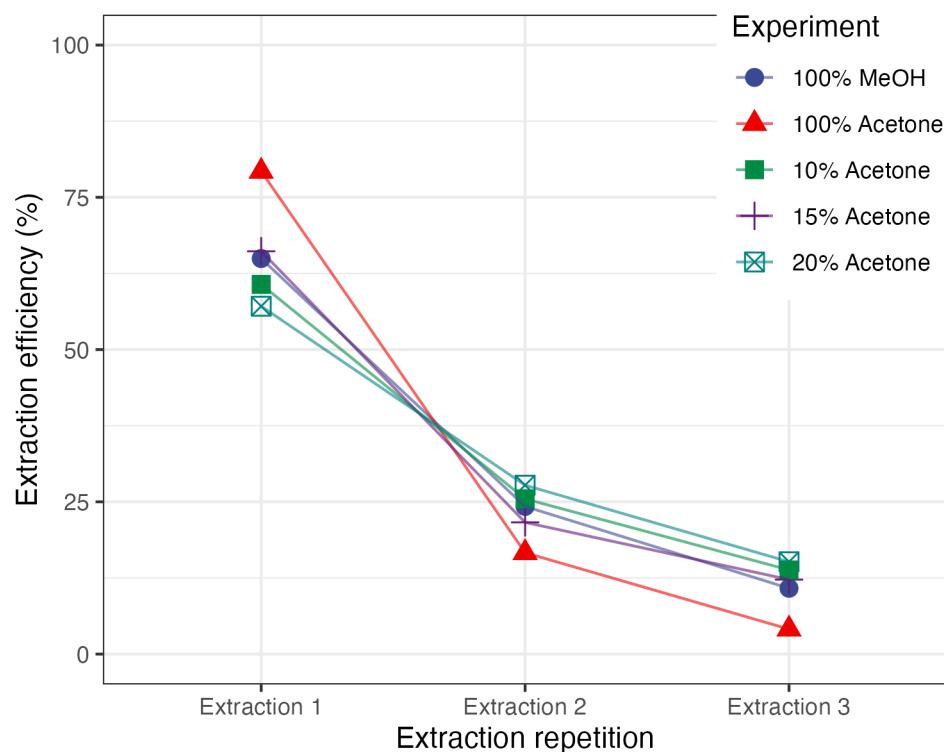
Is the extraction efficiency 100%?

Will acetone break down the cell wall?

Analyte: Chlorogenic acid

Apple accession: DW90

Chlorogenic content: Low



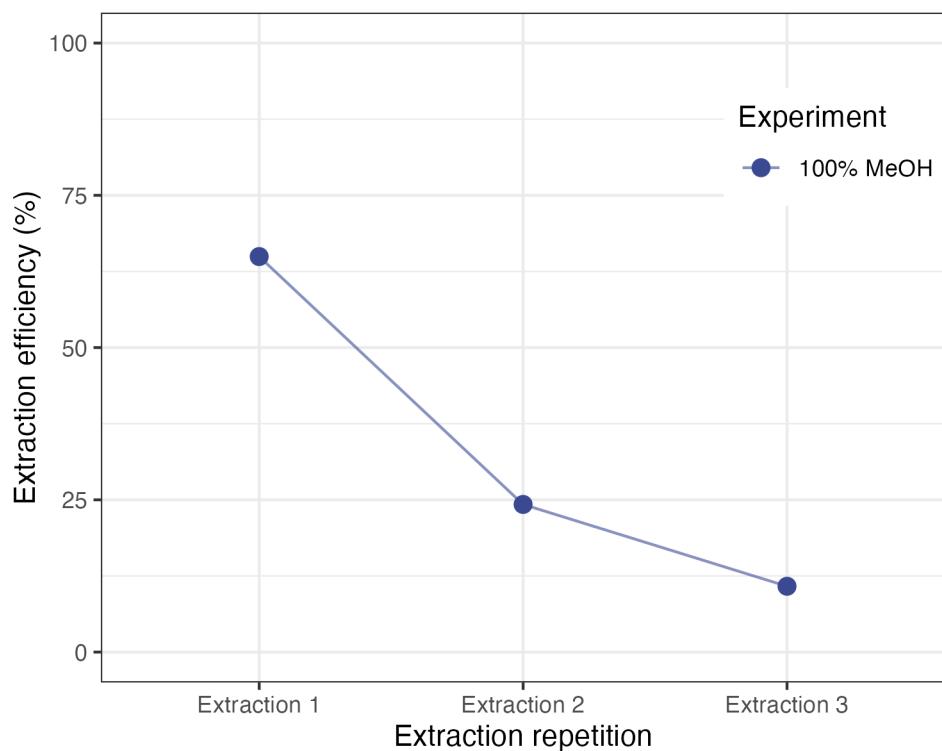
Is the extraction efficiency 100%?

Will acetone break down the cell wall?

Analyte: Chlorogenic acid

Apple accession: DW90

Chlorogenic content: Low

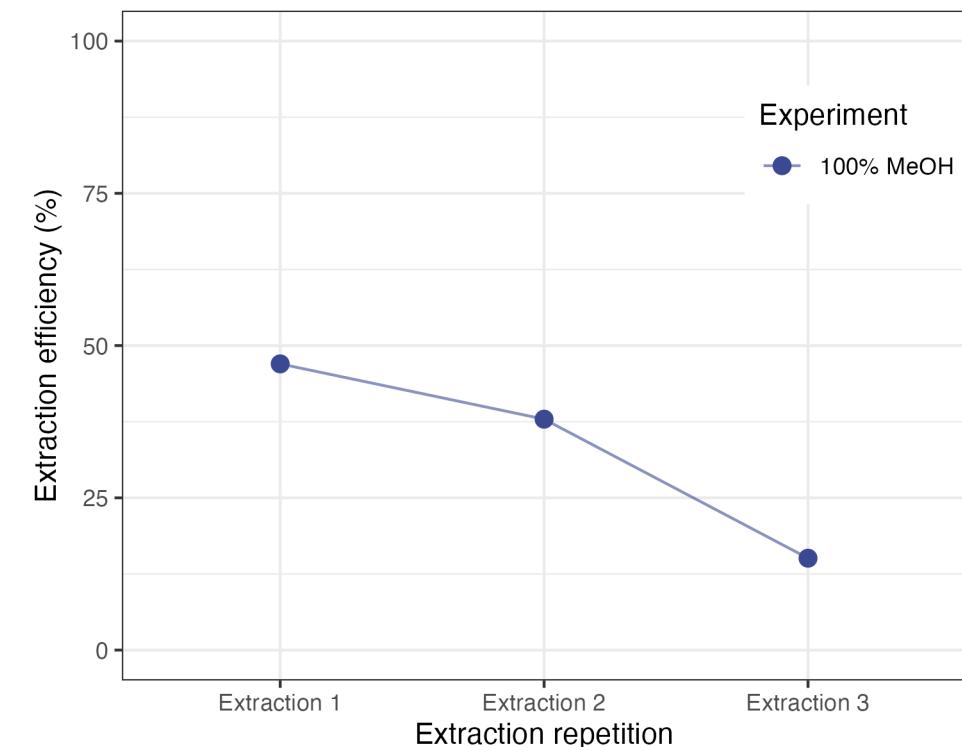


Will FA and HCl break down the cell wall?

Analyte: Chlorogenic acid

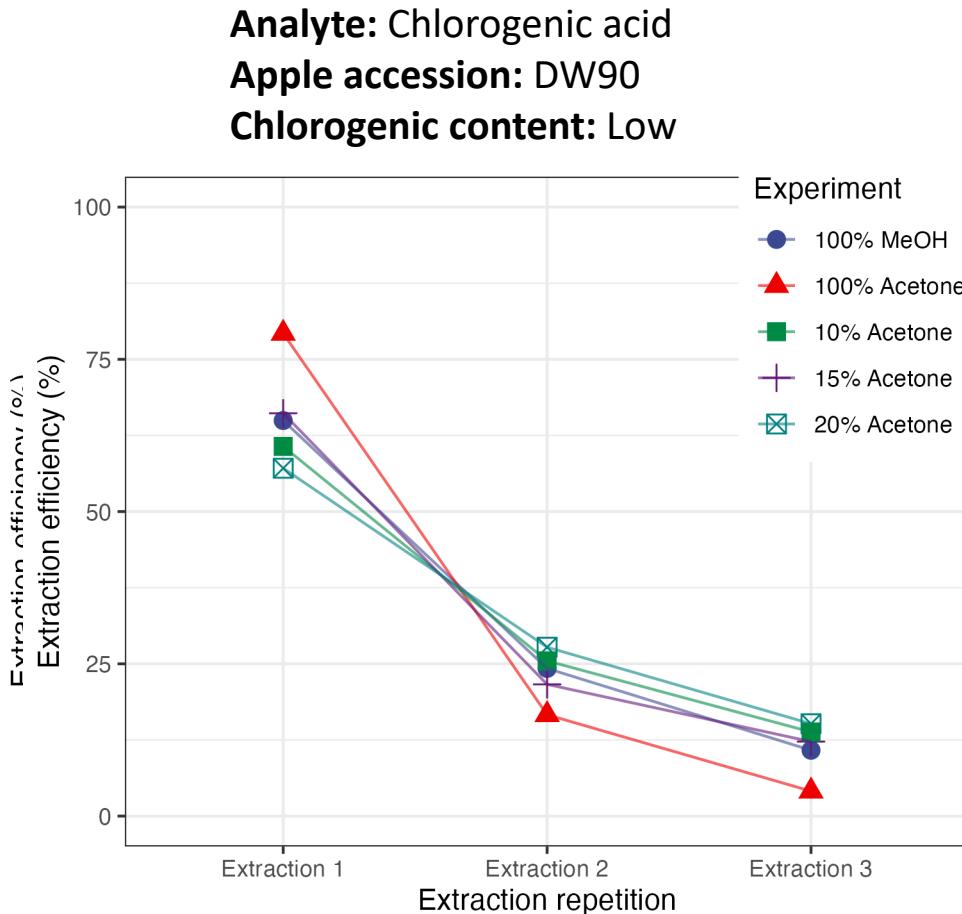
Apple accession: D4097

Chlorogenic content: High

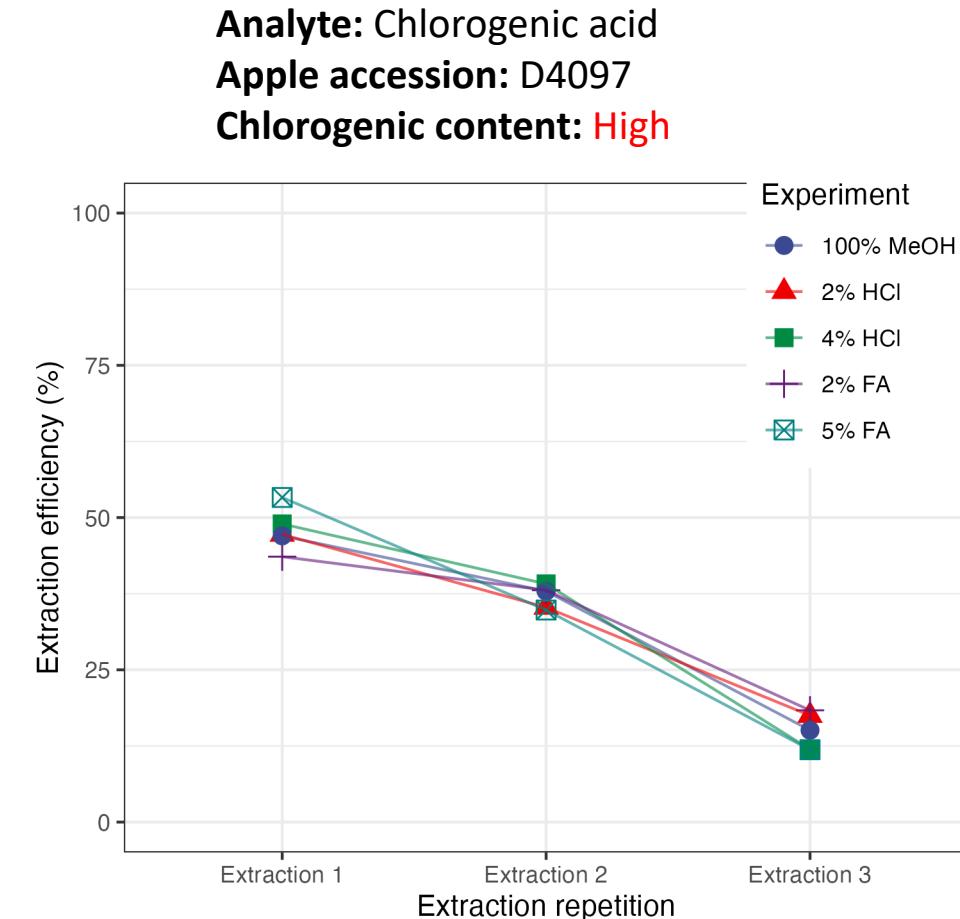


Is the extraction efficiency 100%?

Will acetone break down the cell wall?



Will FA and HCl break down the cell wall?



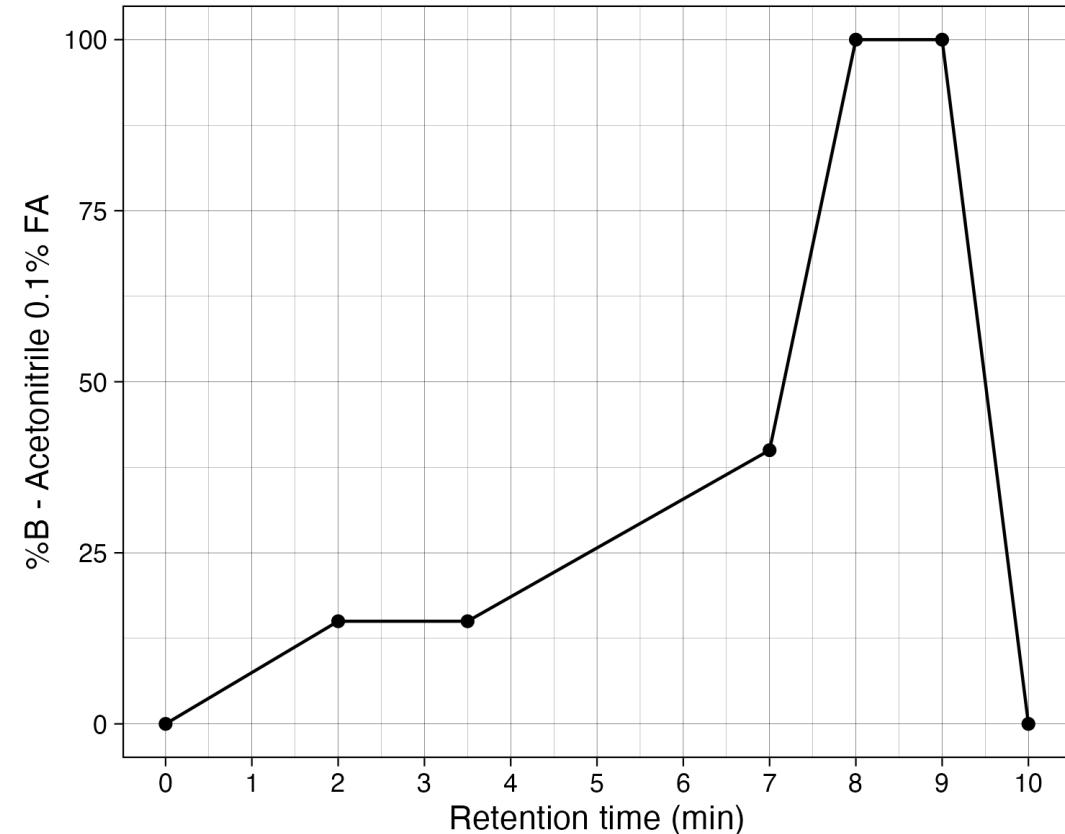
Phenolics quantification method – LC

LC gradient

Time	Flow (mL/min)	%A	%B
0	0.5	100	0
2	0.5	85	15
3.5	0.5	85	15
7	0.5	60	40
8	0.5	0	100
9	0.5	0	100
10	0.5	100	0

A: H₂O + 0.1% Formic Acid

B: Acetonitrile + 0.1% Formic acid

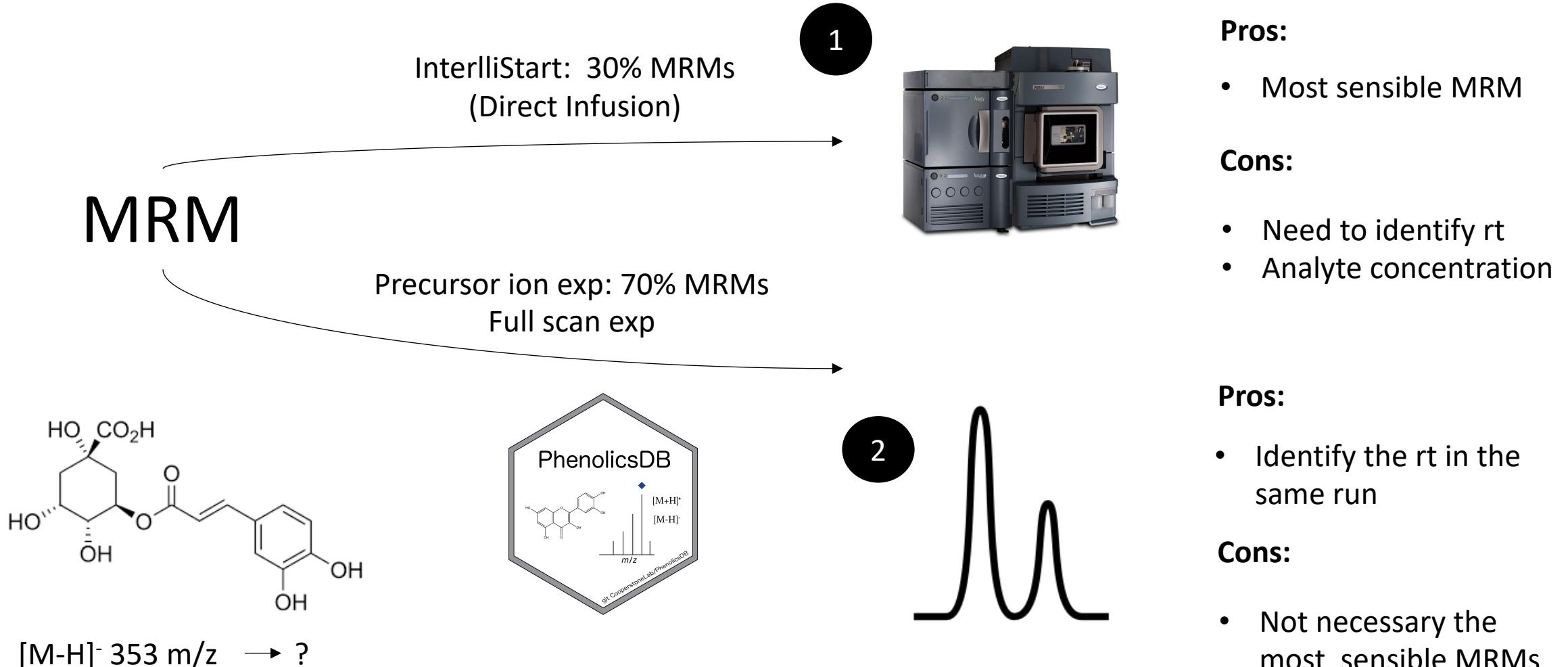


ACQUITY UPLC HSS T3 Column, 100 \AA ,
1.8 μm , 2.1 mm X 50 mm



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Phenolics quantification method – QqQ

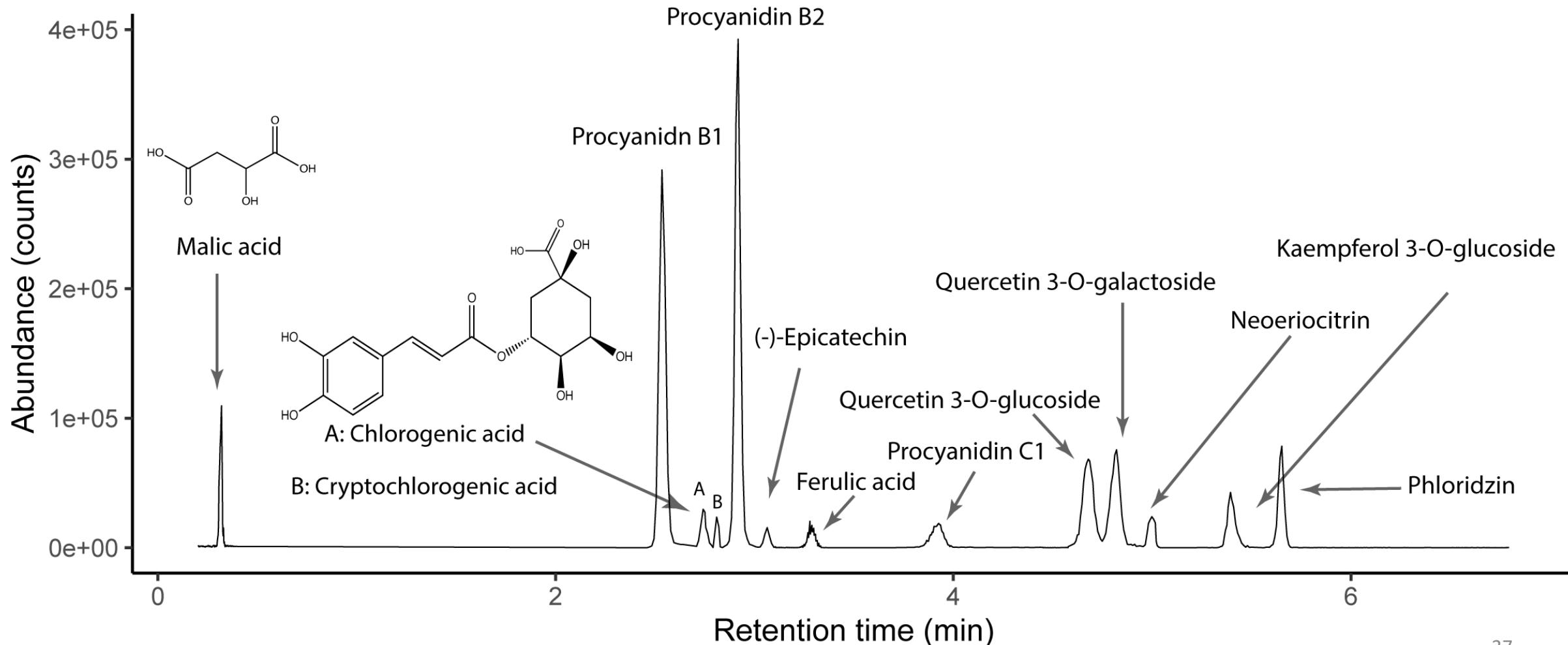


Phenolics quantification method – LC-QqQ

#	Analyte	rt (min)	Formula	Precursor ion	Quant ion	Qual ion
1	Malic acid	0.30	C ₄ H ₆ O ₅	132.5	114.8	88.8
3	Procyanidin B1	2.62	C ₃₀ H ₂₆ O ₁₂	576.7	288.8	406.6
2	Chlorogenic acid	2.70	C ₁₆ H ₁₈ O ₉	353.1	84.9	126.9
5	Cyanidin 3-O-glucoside	2.75	C ₂₁ H ₂₁ O ₁₁	446.7	283.8	182.8
4	Catechin	2.78	C ₁₅ H ₁₄ O ₆	289.0	108.9	124.8
6	Cryptochlorogenic acid	2.86	C ₁₆ H ₁₈ O ₉	353.1	172.9	134.8
8	Procyanidin B2	2.95	C ₃₀ H ₂₆ O ₁₂	577.1	124.9	245.3
7	Cyanidin 3-O-rutinoside	3.04	C ₂₇ H ₃₁ O ₁₅	593.5	285.2	256.2
9	(-)-Epicatechin	3.13	C ₁₅ H ₁₄ O ₆	288.7	244.8	108.9
10	Procyanidin C1	3.30	C ₄₅ H ₃₈ O ₁₈	865.2	261.0	425.0
11	trans-Ferulic acid	4.20	C ₁₀ H ₁₀ O ₄	192.7	133.8	177.8
12	Kaempferol 3-O-rutinoside	4.64	C ₂₇ H ₃₀ O ₁₆	609.1	300.0	151.0
13	Quercetin 3-O-galactoside	4.66	C ₂₁ H ₂₀ O ₁₂	463.0	271.0	300.0
15	Neoeriocitrin	4.79	C ₂₇ H ₃₂ O ₁₅	595.1	151.1	459.1
14	Quercetin 3-O-glucoside	4.85	C ₂₁ H ₂₀ O ₁₂	463.0	271.0	300.0
17	Quercetin 3-O-rhamnoside	5.43	C ₂₁ H ₂₀ O ₁₁	447.1	300.0	281.0
16	Kaempferol 3-O-glucoside	5.44	C ₂₁ H ₂₀ O ₁₂	447.1	255.3	285.1
18	Fisetin	5.74	C ₁₅ H ₁₀ O ₆	285.0	134.8	162.7
19	Phloridzin	5.80	C ₂₁ H ₂₄ O ₁₀	435.1	167.0	273.0

Table. MRM method, analytes are sorted by rt. Analytes colored in red represents internal standards

Phenolics quantification method – LC-QqQ



Acknowledgements

Jessica Cooperstone, PhD
Advisor

Cooperstone lab

- Jordan Hartman (JL)
- Daniel Do
- Maria Shalola
- Lydia Balogh
- Aaron Wiedemer

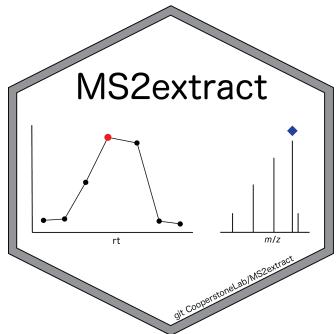
SAC:

- Diane Miller
- Jonathan Fresnedo Ramirez



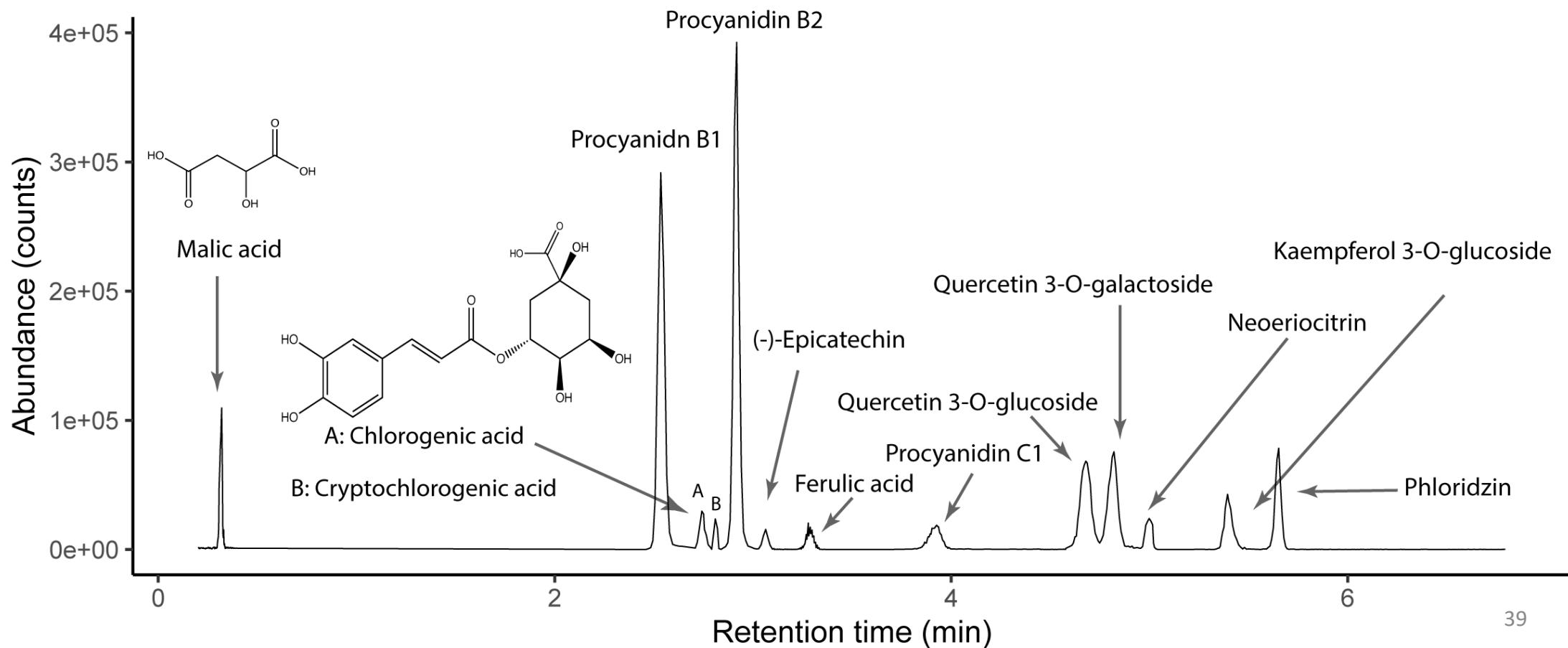
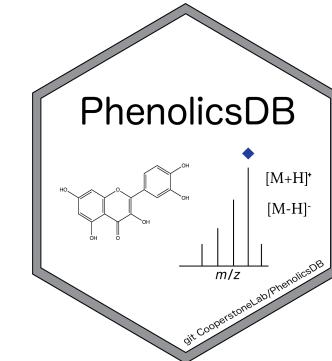
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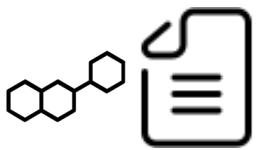
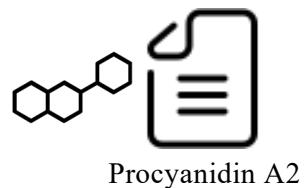
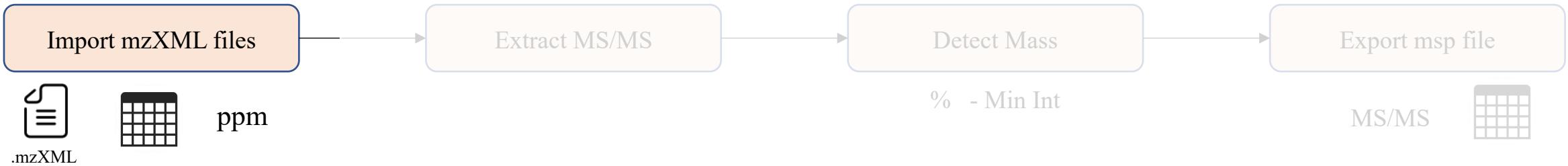




Thank you

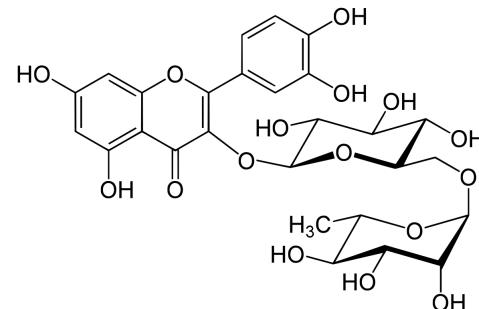
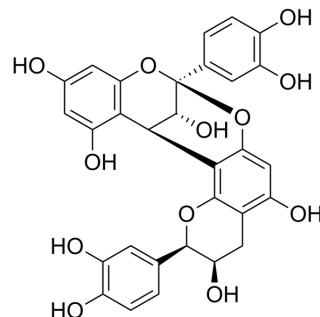
Questions?

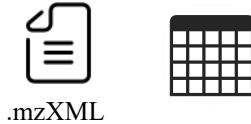




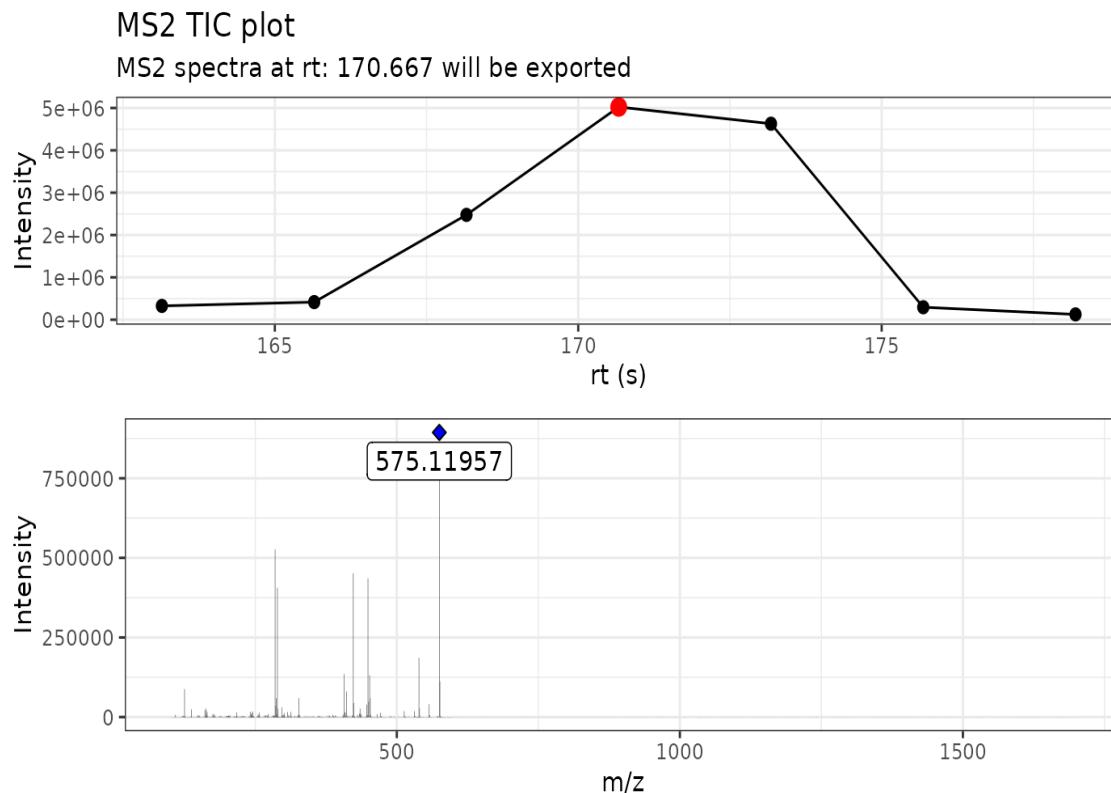
+

Name	Formula	Ionization_mode	min_rt	max_rt
Procyanidin A2	C30H24O12	Negative	163	180
Rutin	C27H30O16	Negative	162	171

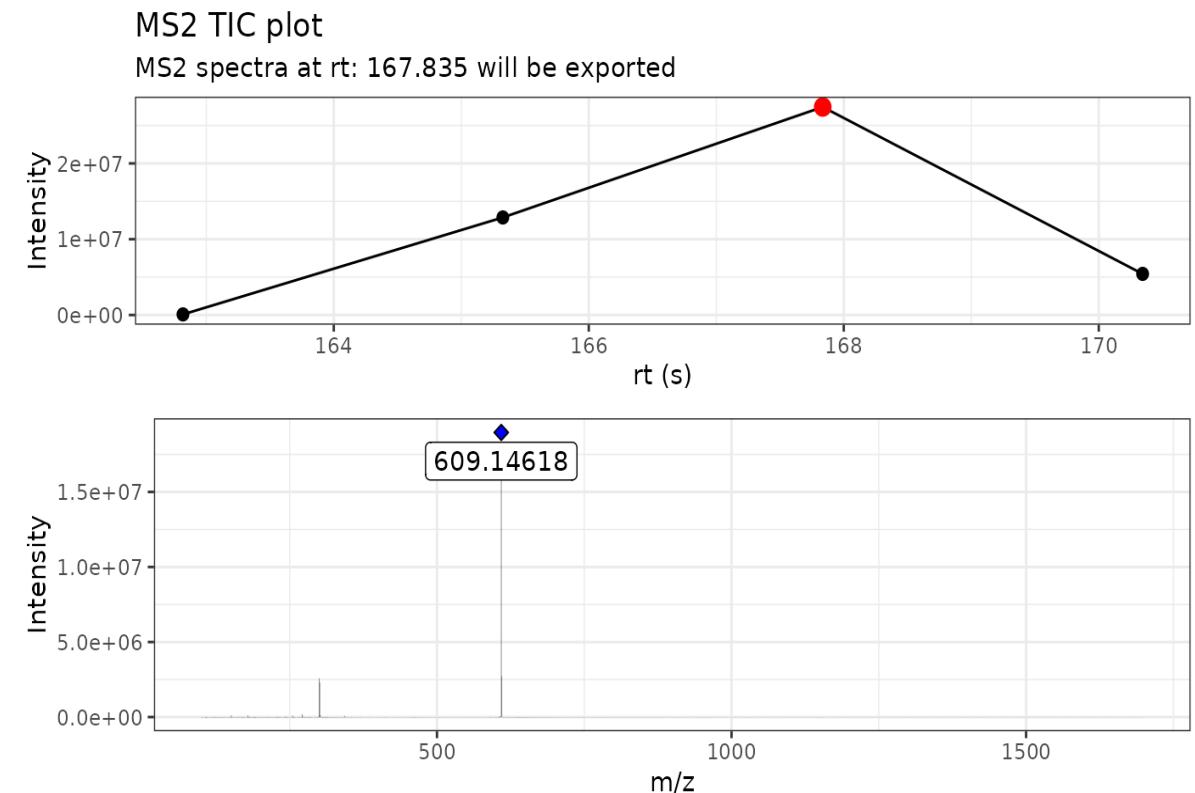




Procyanidin A2



Rutin



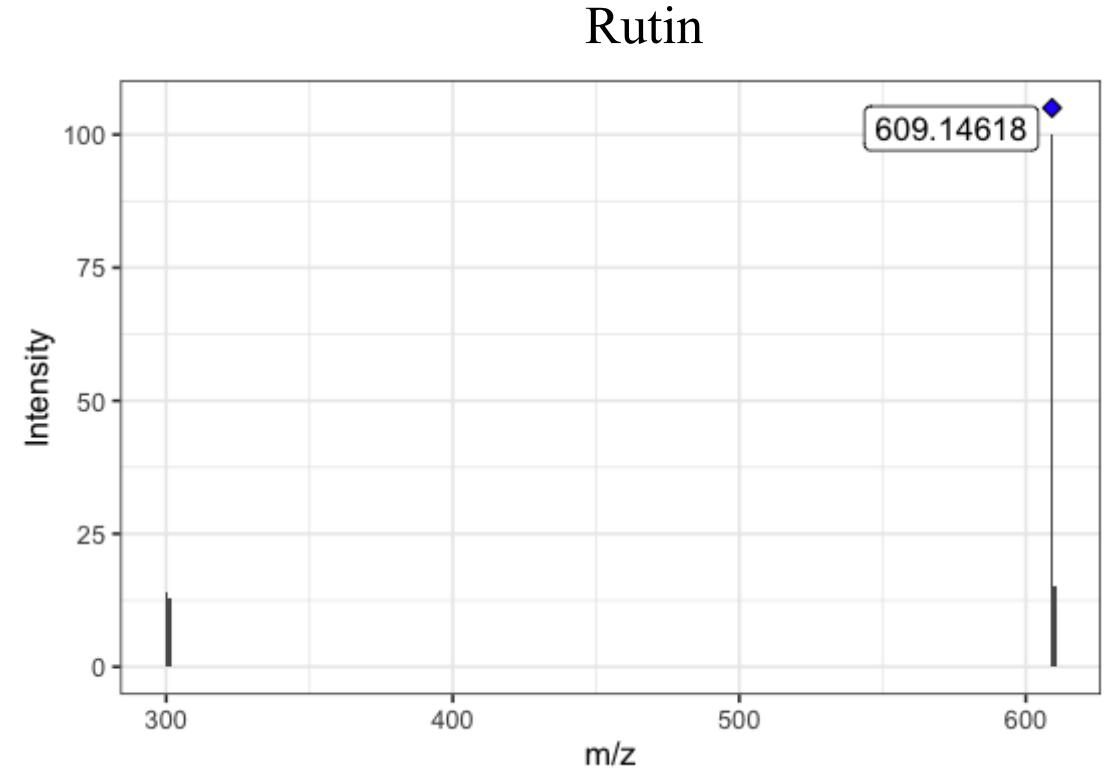
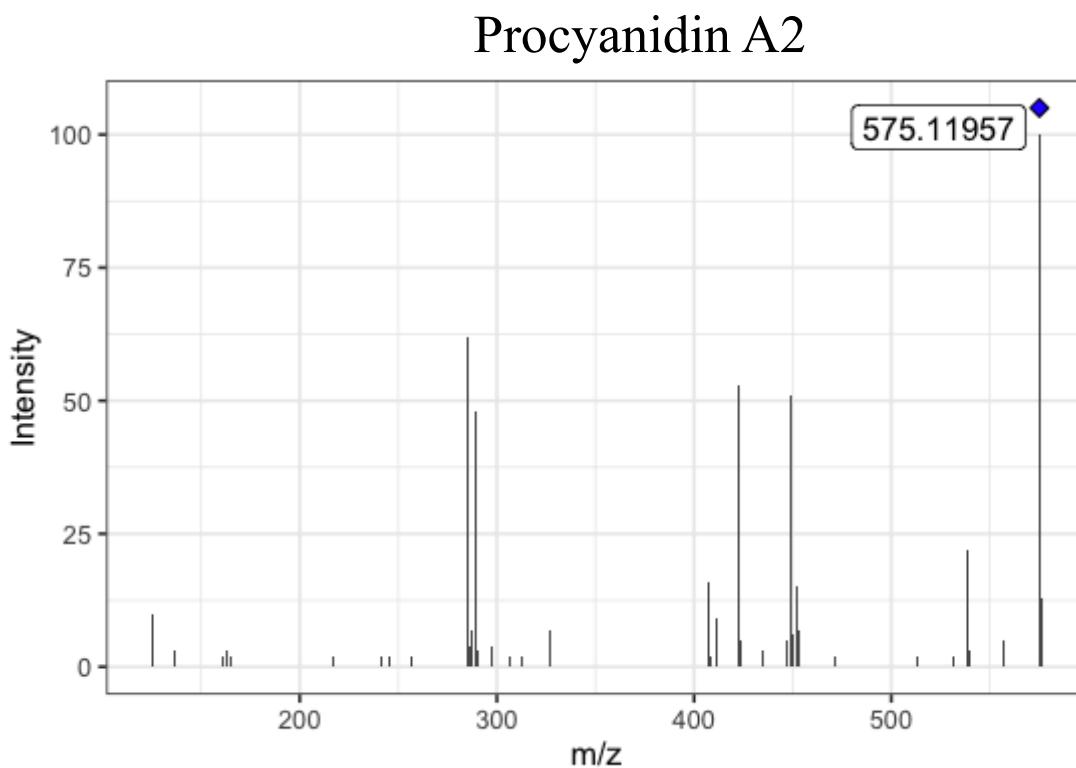


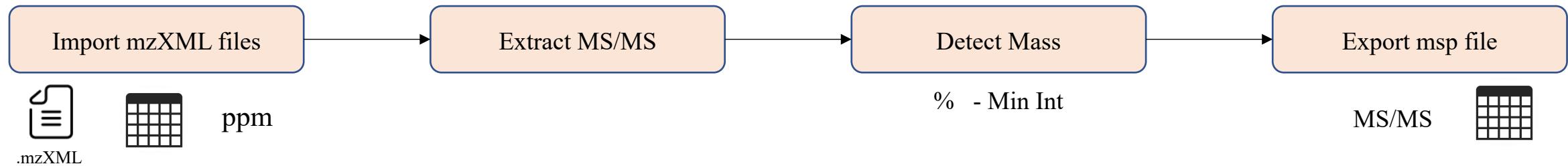
.mzXML



ppm

% - Min Int





NAME: Rutin
PRECURSORMZ: 609.146179199219
PRECURSORTYPE: [M-H]
FORMULA: C₂₇H₃₀O₁₆
RETENTIONTIME: 167.835
IONMODE: Negative
COMMENT: Spectra extracted with MS2extract R package
INCHIKEY: IKGXIBQEEMLURG-NVPHPEKSA-N
SMILES: CC1C(C(C(C(01)OCC2C(C(C(C(02)OC3=C(OC4=CC(=CO4)C3)O)C2)O)O1)O
CCS:
COLLISIONENERGY: 20 eV
INSTRUMENTTYPE: Q-TOF 6546
Num Peaks: 4
300.02805 14
301.03575 13
609.15002 100
610.15114 15