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Scalable Anthocyanin Extraction and Purification Methods for Industrial Applications

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EPSRC
Engineering and Physical Sciences Research Council

@SanniaF @keracol

Aims

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- Scalable.
- Economically viable.
- Activity/ function driven research.
- Natural product cosmetics.
- Natural hair dyes.
- Natural food colourants.
- Full characterisation of natural extracts.
- Generating multiple product streams from single source.
- Sustainable materials.
- Safe.

www.cosmeticsbusiness.com
www.n-r-c.com
www.healthcare-online.org

Extraction of anthocyanins from blackcurrant waste

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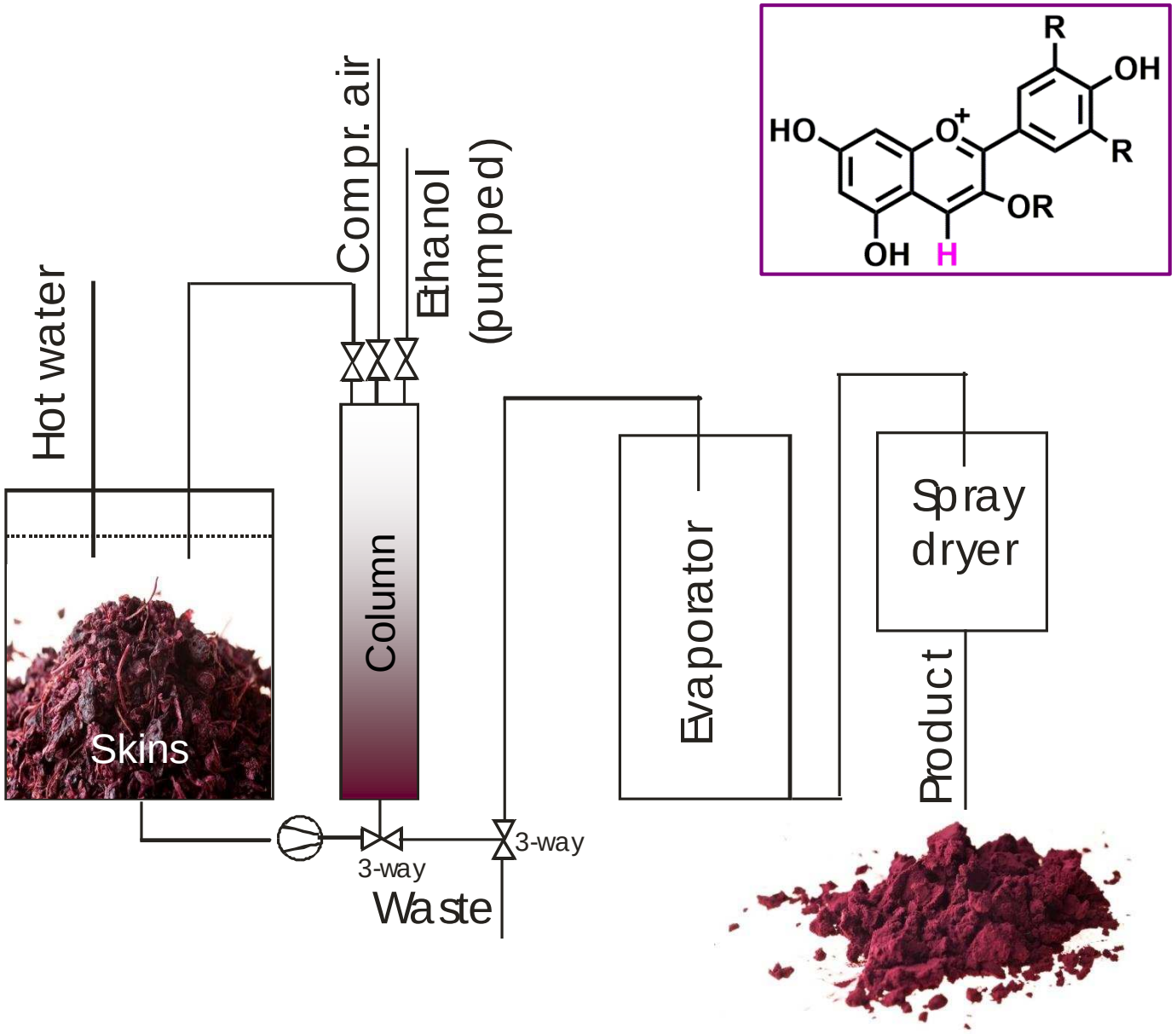
- Blackcurrant grown in the UK.
- Rich in coloured anthocyanins.
- Also contains neutral polyphenols.

- Ribena uses blackcurrant for concentrates and drinks.
- They are left with tons of blackcurrant skins in waste.
- We wanted to find a use for this waste.

Extraction-Purification

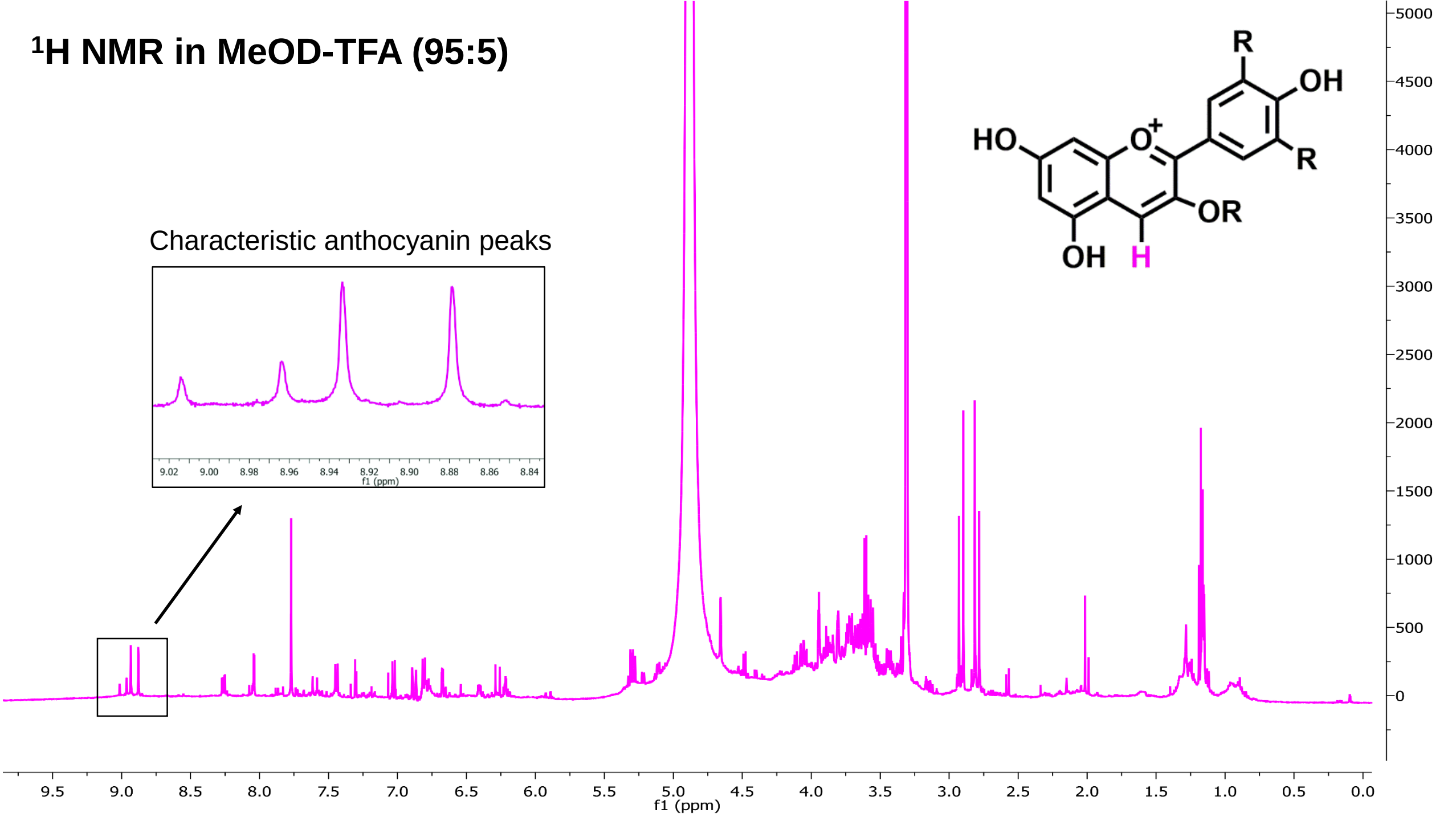


Industrial-scale process

- Extract from berry waste.
- Fruits are used in juice production.
- Only waste skins are used to produce the extract.
- Sustainably sourced.
- Acidic aqueous extraction of dried skins
- Concentration using Solid Phase Extraction (SPE).
  - resin removes free sugars and small organic acids
- Ethanol elution of polyphenols retained by resin.
- 20 litres extract successfully produced.

^{1}H NMR in MeOD-TFA (95:5)

Characteristic anthocyanin peaks



Elution profile of anthocyanins in the blackcurrant extract at 520 nm

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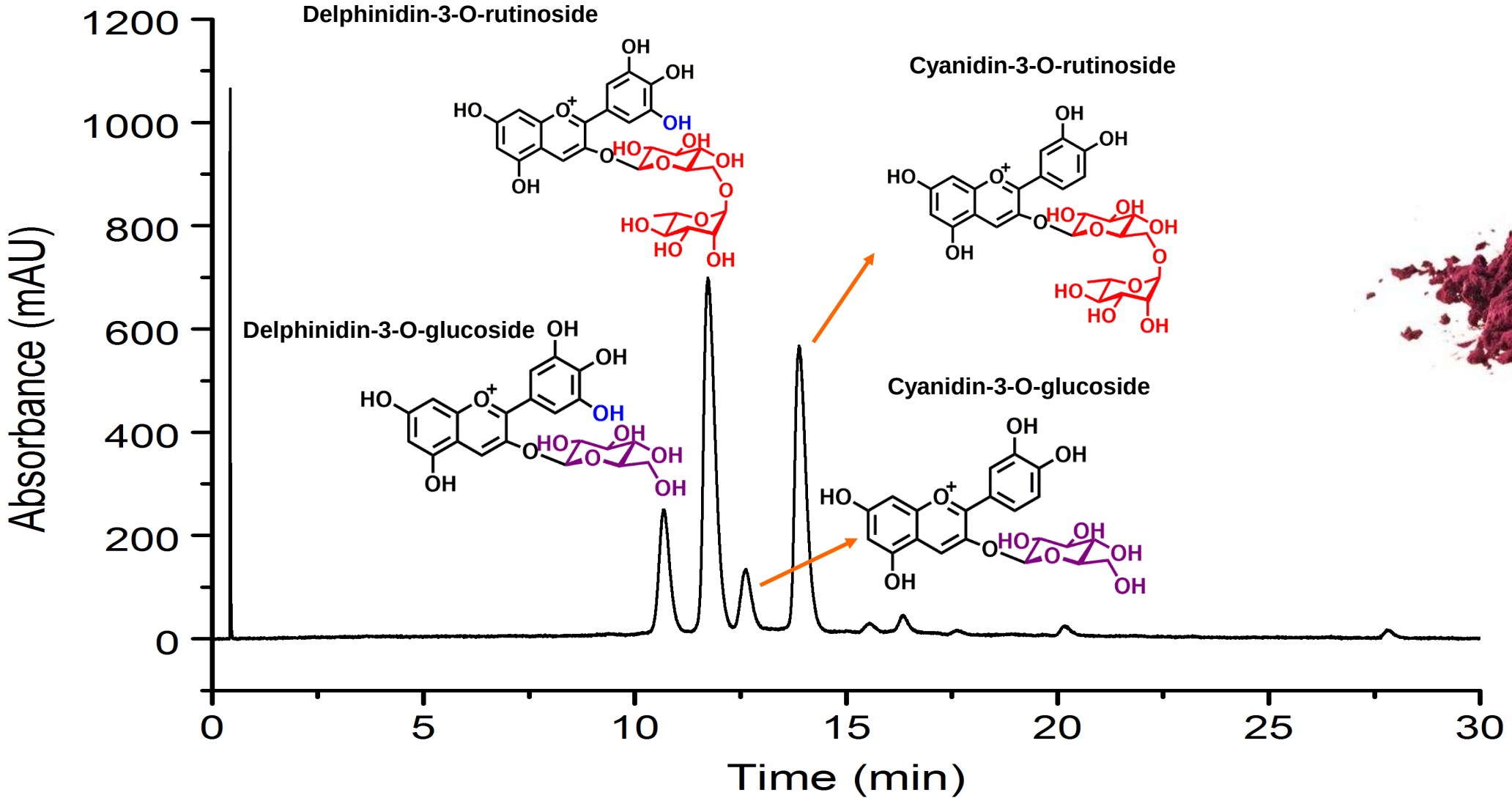

Identification of neutral polyphenols in the extract

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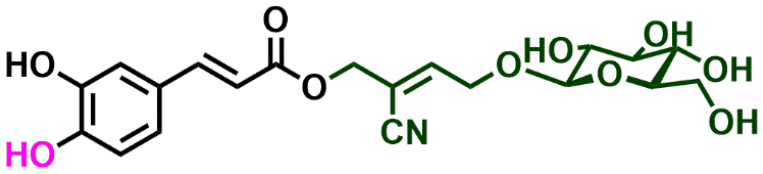
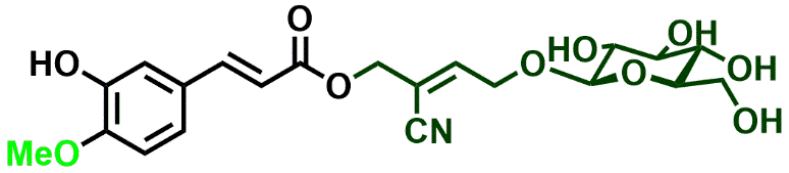
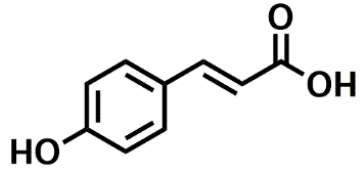


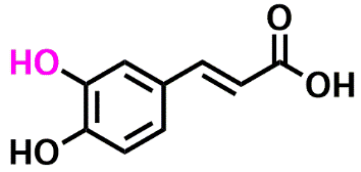
Nigrumin-p-coumarate



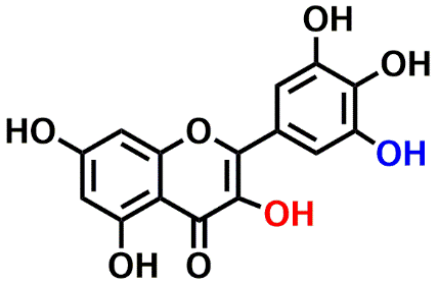
Nigrumin ferulate



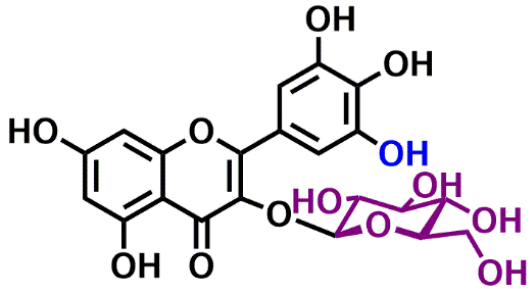
p-Coumaric acid



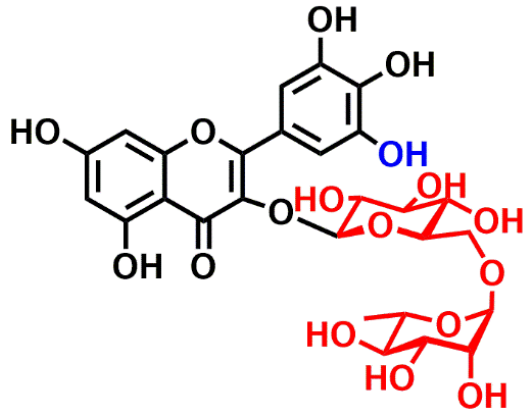
Caffeic acid



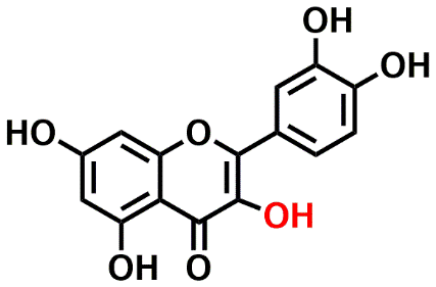
Myricetin



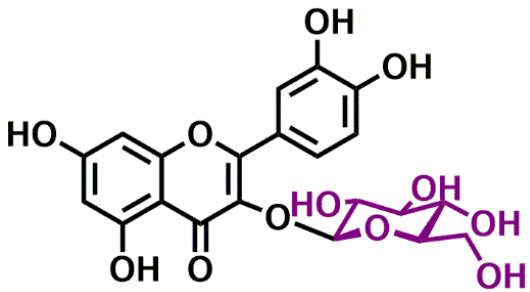
Myricetin-3-O-glucoside



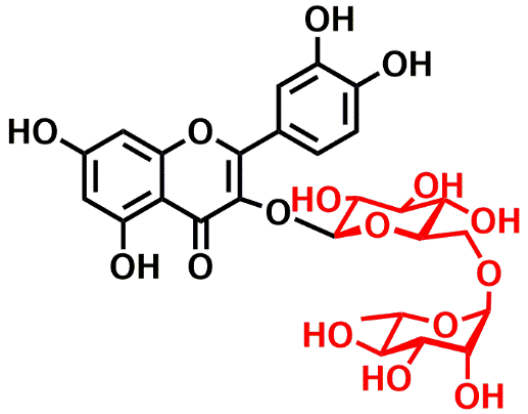
Myricetin-3-O-rutinoside



Quercetin



Quercetin-3-O-glucoside



Quercetin-3-O-rutinoside

Chemical Composition of Blackcurrant Extract

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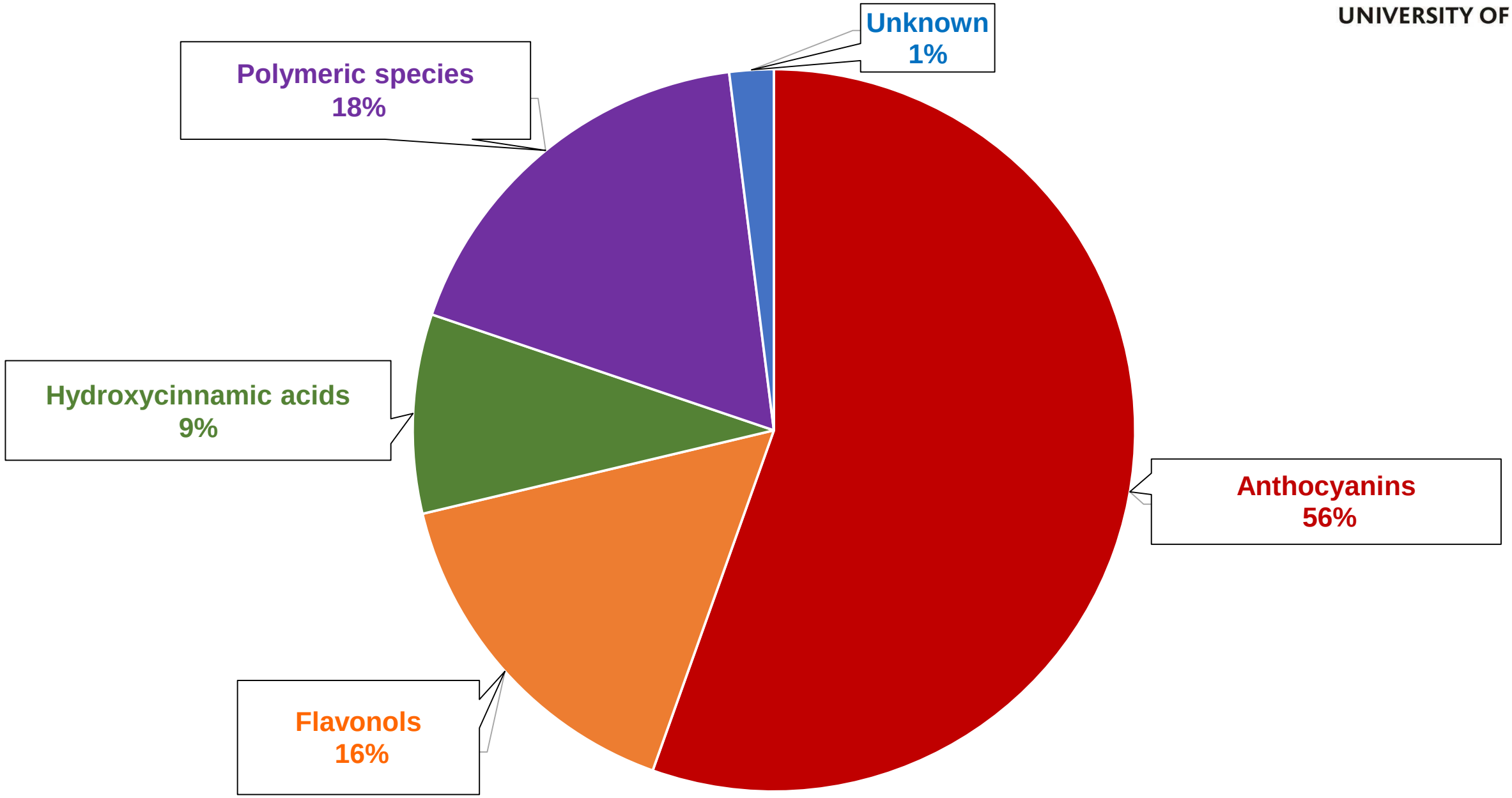

Aqueous-organic partitioning

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- Semi-purify the extract using a scalable and economically suitable method.
- Anthocyanins are very soluble in water due to cationic nature and glycosylation.
- Neutral polyphenols are relatively less polar and expected to migrate to the organic solvent.
- Sugar moieties can enhance water solubility of neutral polyphenols.
- Isopropyl acetate and ethyl acetate were used as organic phase.

Sequential Extractions

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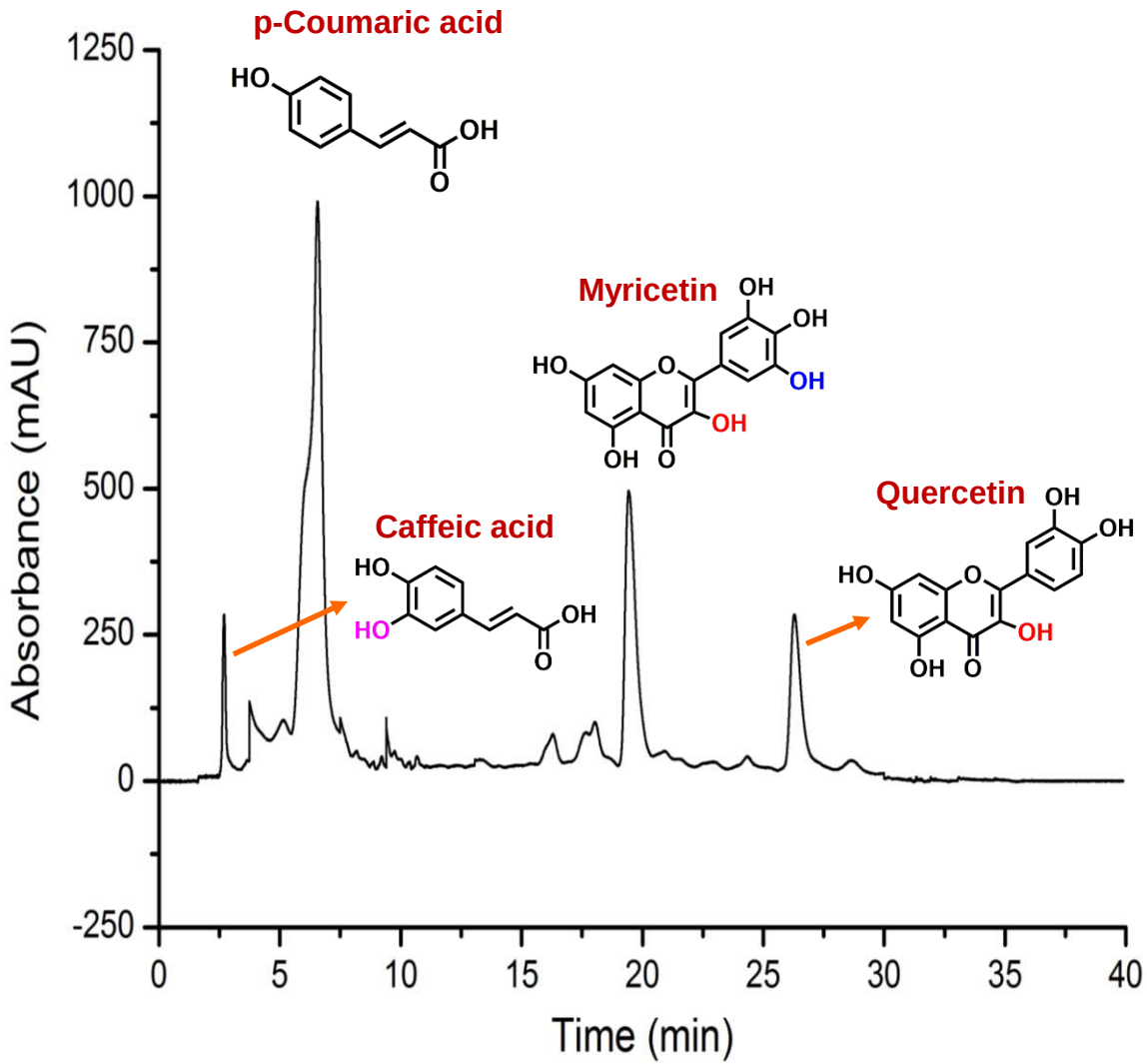
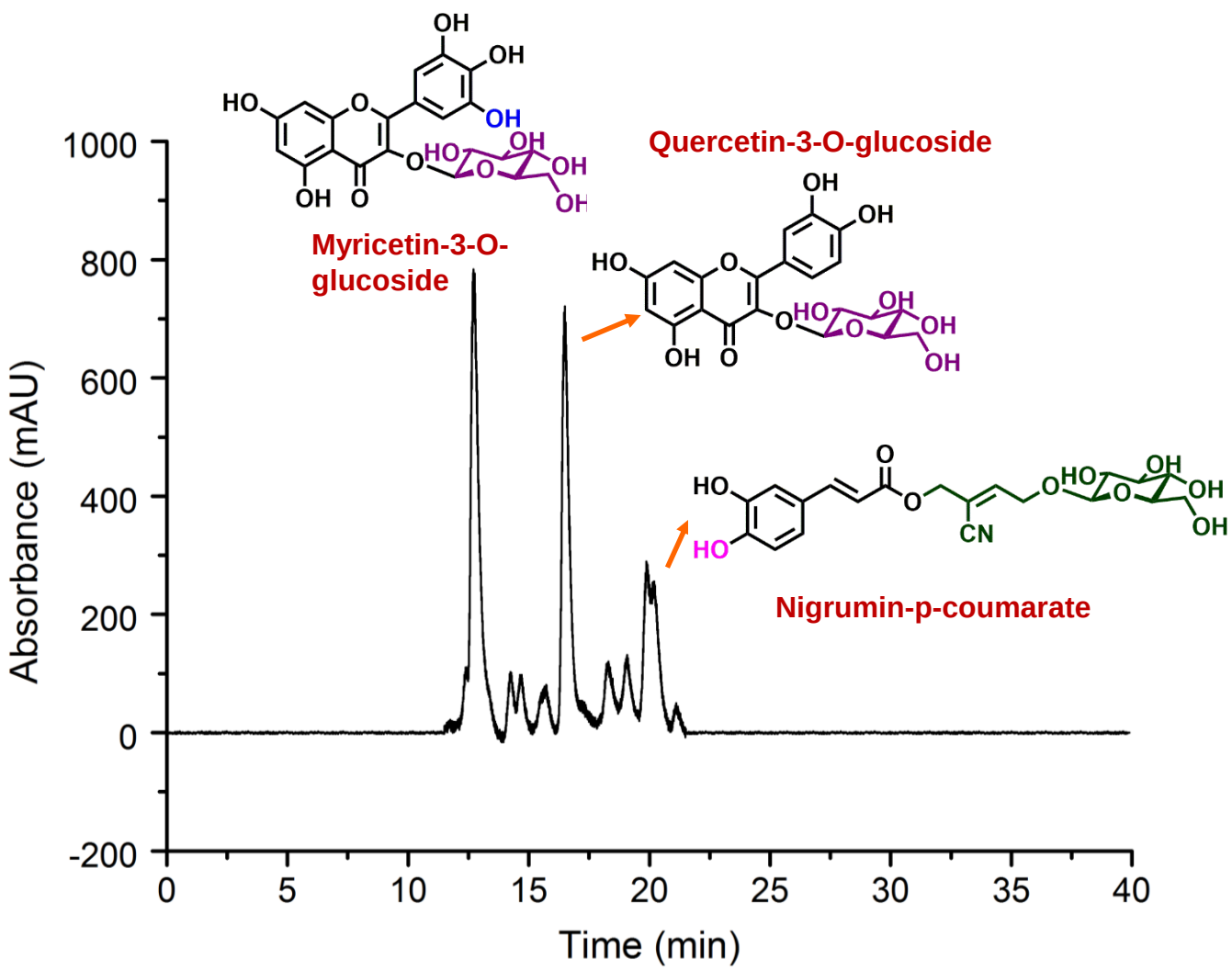
- Isopropyl acetate showed selectivity towards flavonols aglycones.
- The subtle difference in polarity of isopropyl and ethyl acetate was exploited.
- Aqueous blackcurrant extract (pH 1.8) was partitioned against hexane, isopropyl acetate and ethyl acetate sequentially.
- All the layers were analysed through analytical HPLC as well as ^{1}H NMR.
- Isopropyl acetate extracted the aglycones only whereas mono-glycosides migrated into the ethyl acetate layer.
- Aqueous layer retained all the anthocyanins as well as rutinosides of quercetin and myricetin.

Elution profile of isopropyl acetate extract at 325 nm



Elution profile of ethyl acetate extract at 350 nm

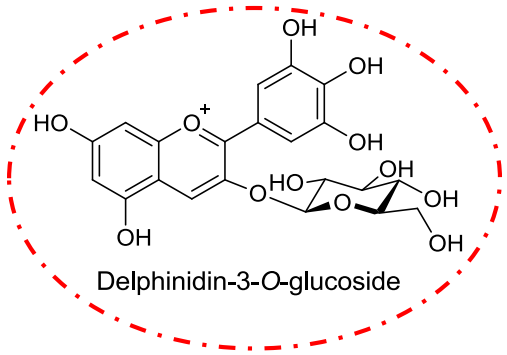


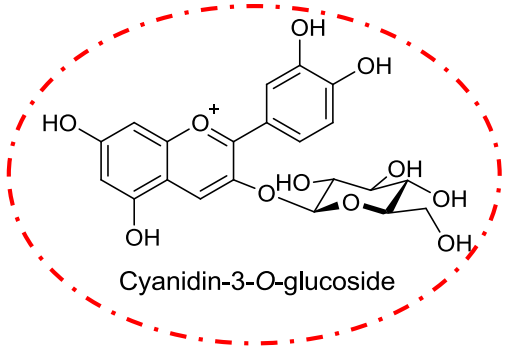
Liquid-liquid partitioning

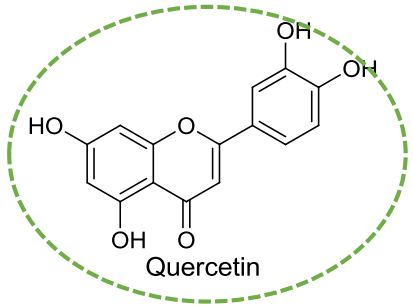
ethyl acetate

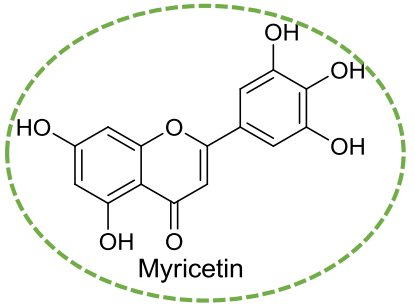
ⁱpropyl acetate

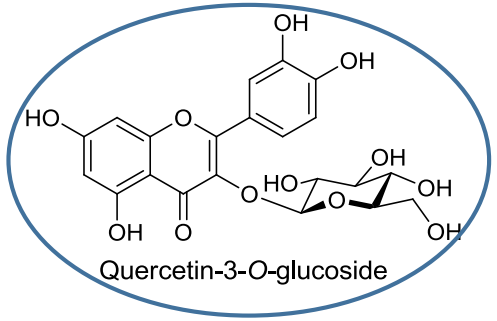
aqueous

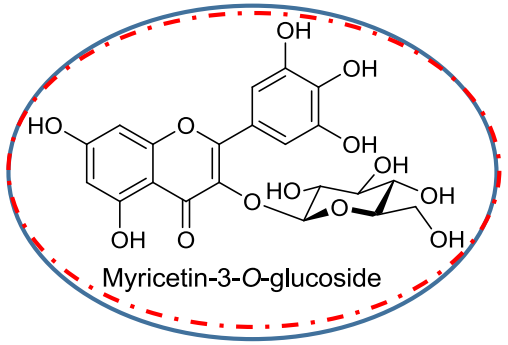


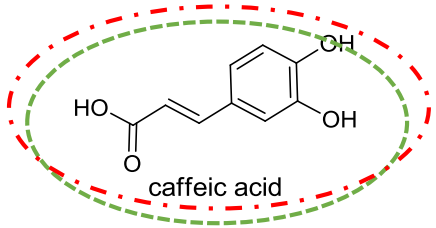


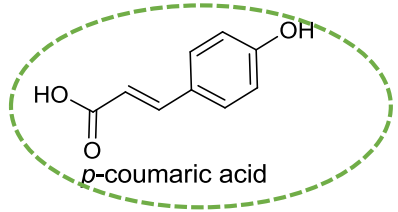


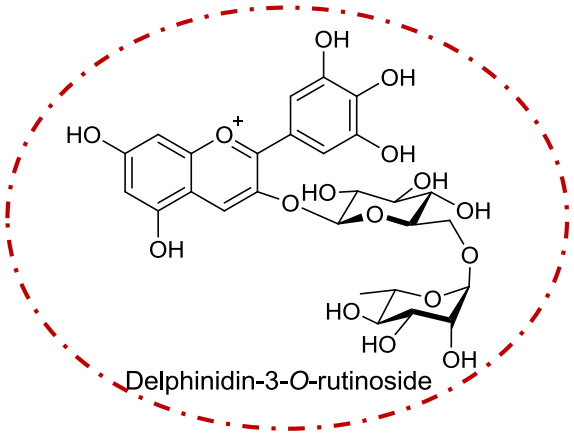


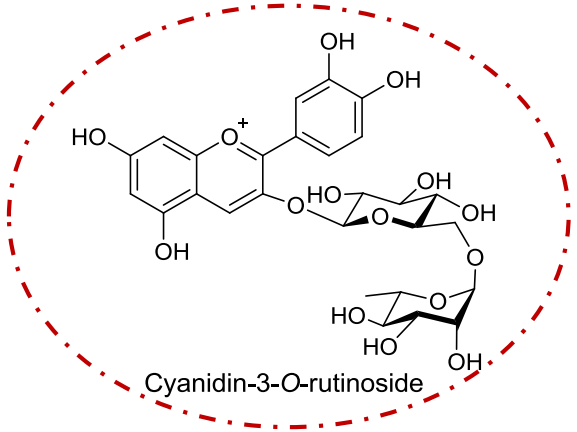


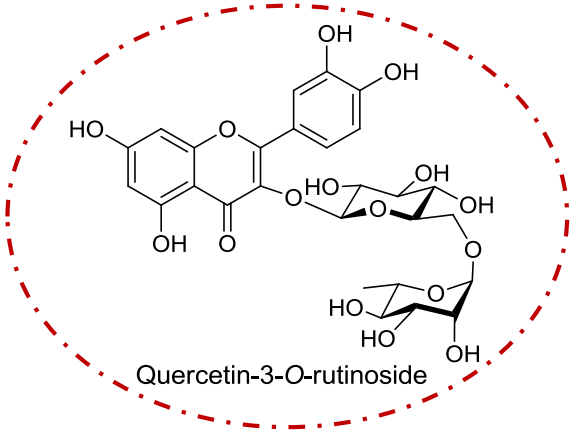


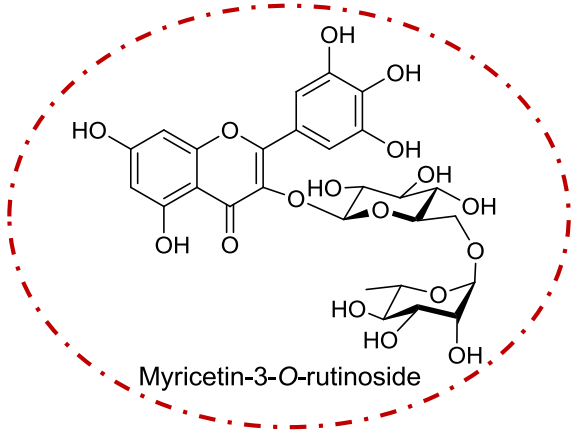












Equilibrium Forms of Anthocyanins

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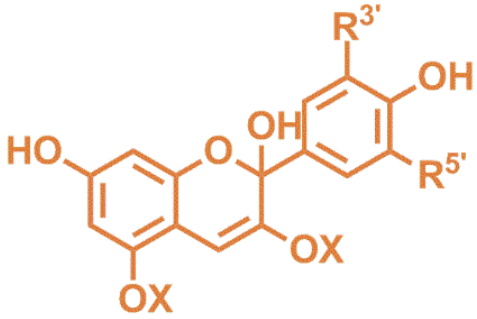
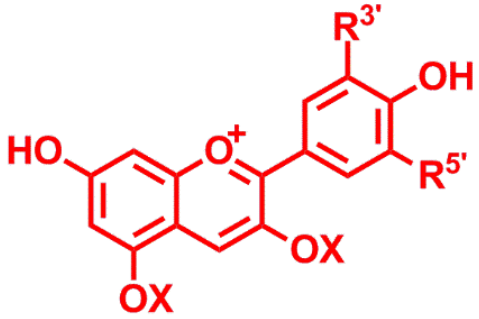
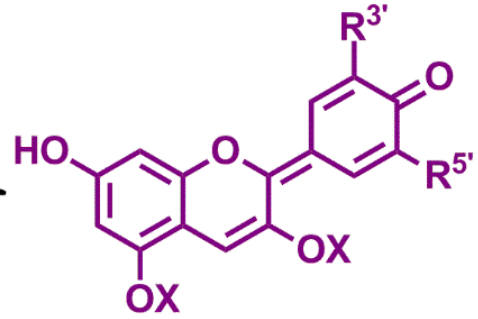


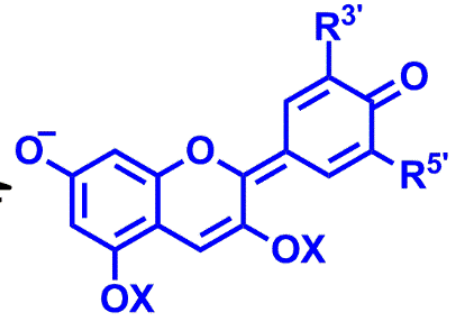
Hemiacetal, B_2



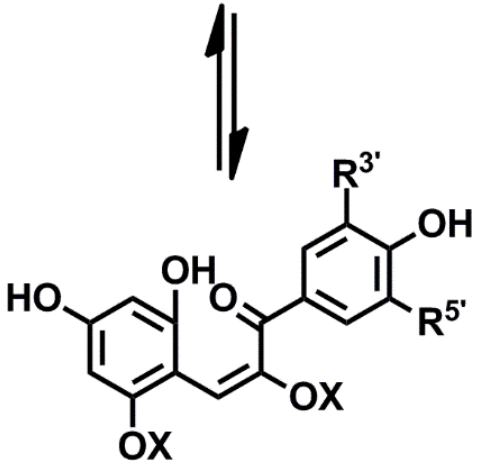
Flavylium cation, AH^+



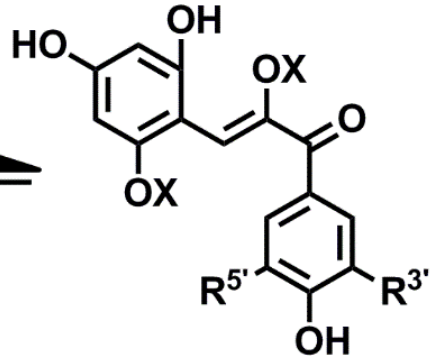
Quinoidal base, $A_{4'}$



Quinoid base, $A_{4'7}$



Cis-chalcone, C_E



Trans-chalcone, C_Z

Chem. Soc. Rev., 2012, **41**, 869–908

pH-Controlled aqueous-organic extractions

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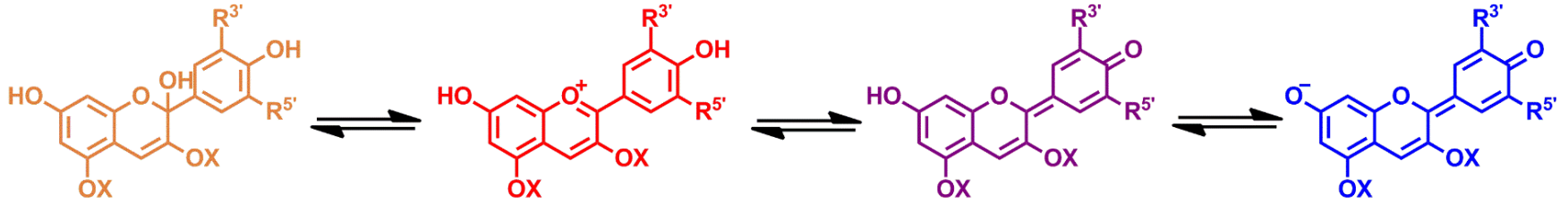
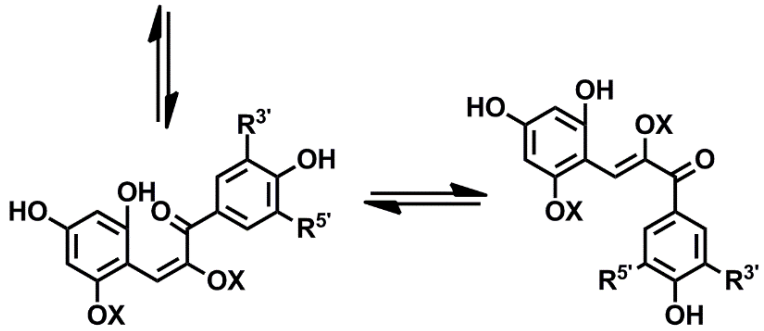
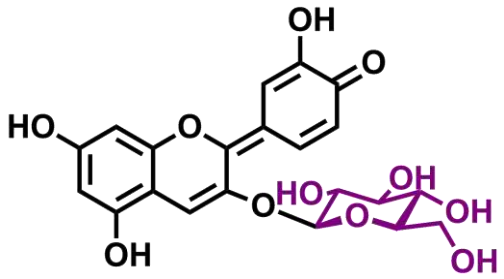
- Anthocyanins can exist in neutral equilibrium forms at higher pHs.
- The mole fraction of each form varies with the substitution pattern.
- Can we manipulate these forms to separate rutinosides from glucosides?
- Around neutral pH anthocyanins can exist in quinoidal base form.
- **The aqueous extract was partitioned against ethyl acetate at pH 1.8, 6.3 and 12.1.**



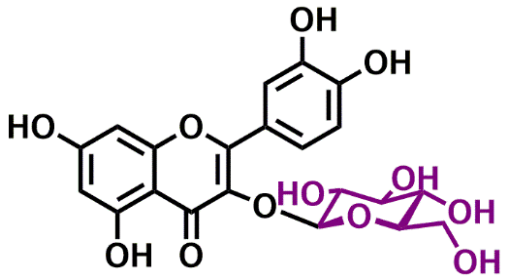
Hemiacetal, B_2 | Flavylium cation, AH^+ | Quinoidal base, $A_{4'}$ | Quinoid base, $A_{4'7}$



Cis-chalcone, C_E | Trans-chalcone, C_Z

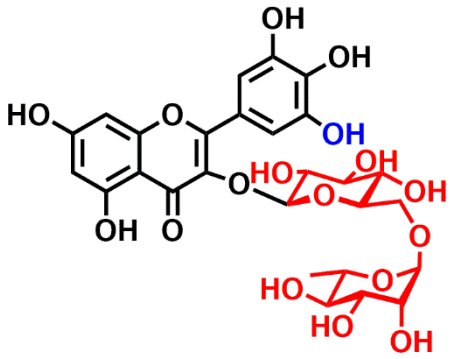


COG quinoidal base form



QOG

Ethyl acetate extracts in CD_3OD/TFA after pH controlled extractions

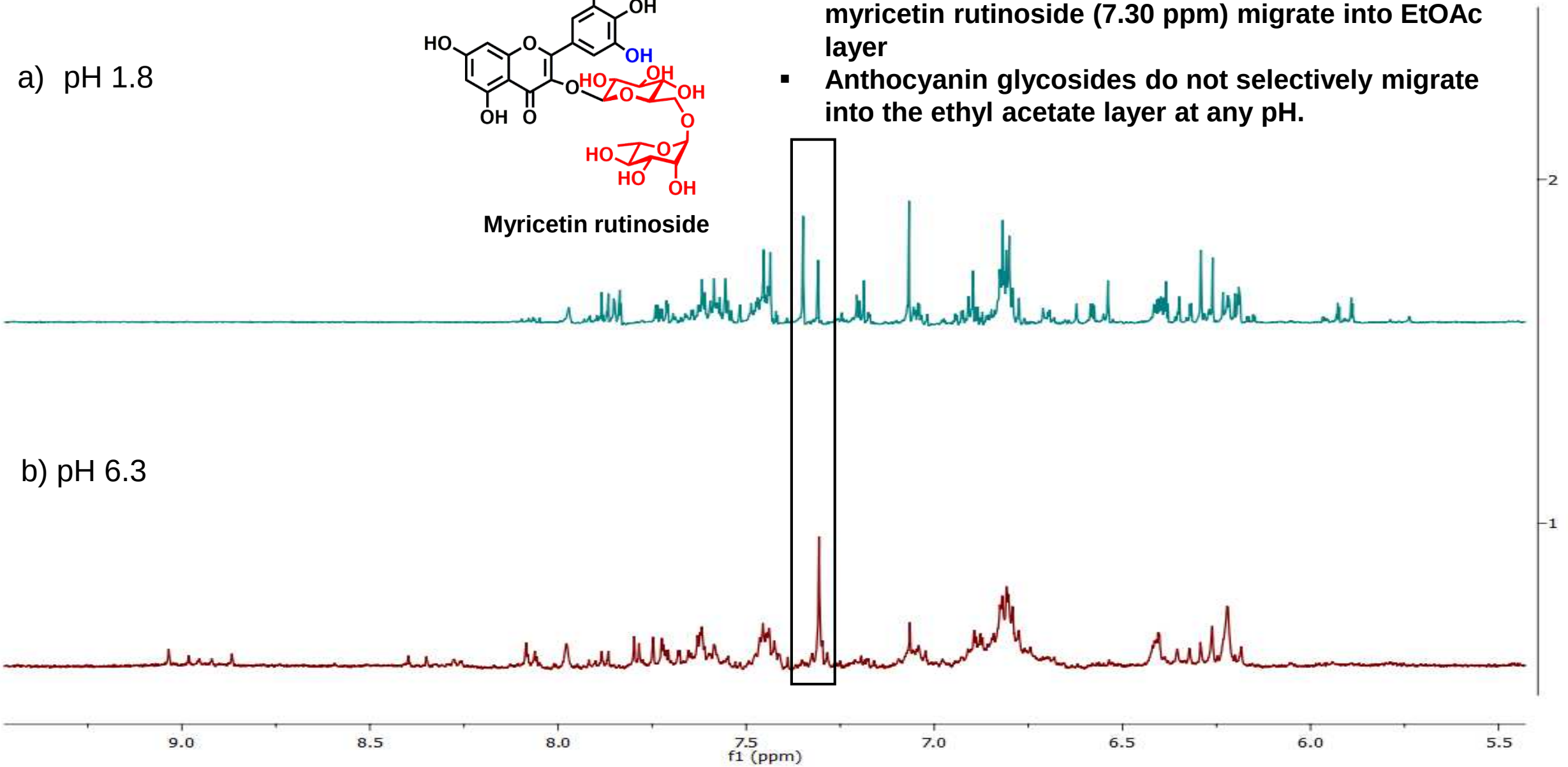


Myricetin rutinoside

- **At pH 6.3: quercetin rutinoside (7.52 ppm) and myricetin rutinoside (7.30 ppm) migrate into EtOAc layer**
- **Anthocyanin glycosides do not selectively migrate into the ethyl acetate layer at any pH.**

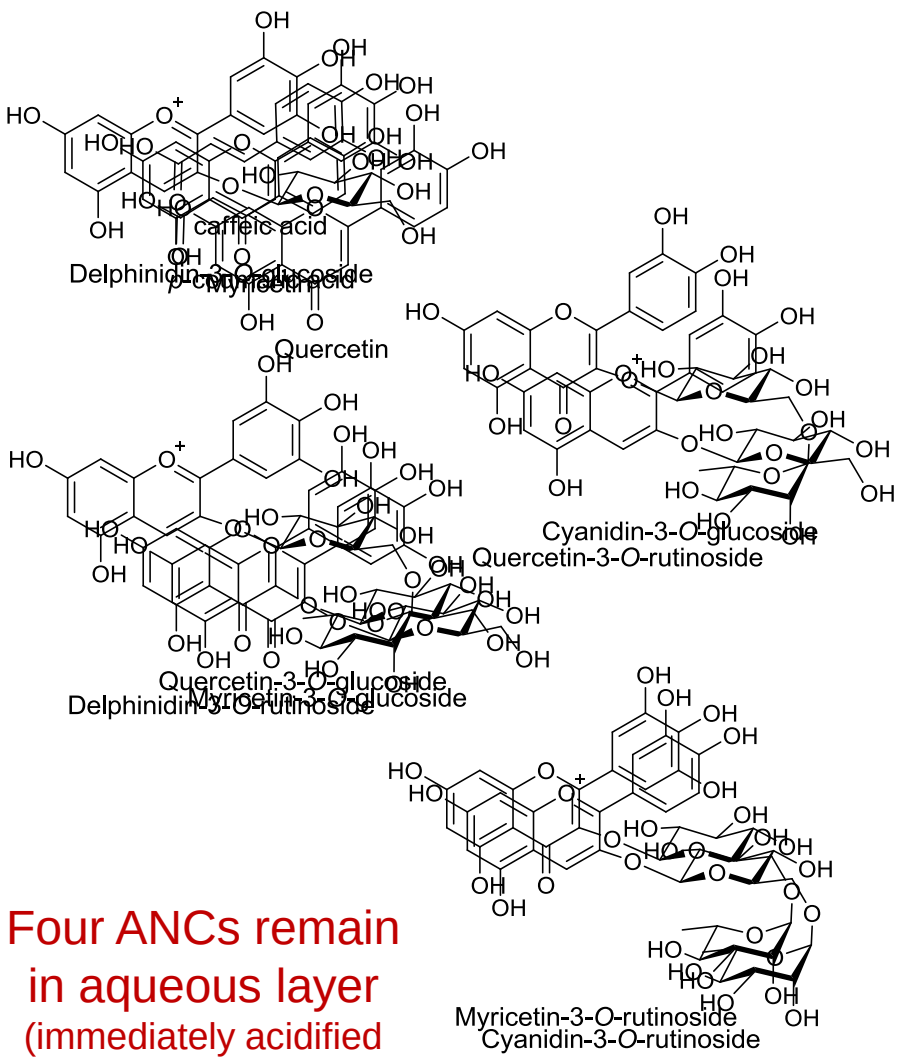
a) pH 1.8

b) pH 6.3



Sequential liquid-liquid partitioning with pH changes

BC extract comprises 56% ANC and 25% other polyphenols



Four ANCs remain in aqueous layer (immediately acidified and freeze dried)

Myricetin and quercetin rutinosides removed with **EtOAc at pH 6.3**

Trace ANC

EtOAc at pH 1.8 selectively removes myricetin + quercetin glucosides

^{i}PrOAc at pH 1.8 removes hydroxycinnamic acids and myricetin + quercetin aglycons

Conclusions

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- Blackcurrant extract post-SPE can be purified using sequential liquid-liquid and pH change extractions.
- The purification methods are scalable.
- Three product streams.
- One containing four anthocyanins (responsible for colour) in a process that is industrially scalable.
- The two organic extracts contain known antioxidants which can be used in activity-based products.
- The extracts contain polyphenols of slightly varied polarity and so can be formulated in different products.
- The established methods have been successfully applied to other natural extracts as well.

Acknowledgements

- University of Leeds for the opportunity.
- EPSRC for funding.
- Prof Chris Rayner and Dr Richard Blackburn for their continuous guidance and support.
- Technical staff, Simon Barrett and Martin Huscroft for helping me with NMR and HPLC.
- Rest of the group for their suggestions and support.

- Thank you for listening!

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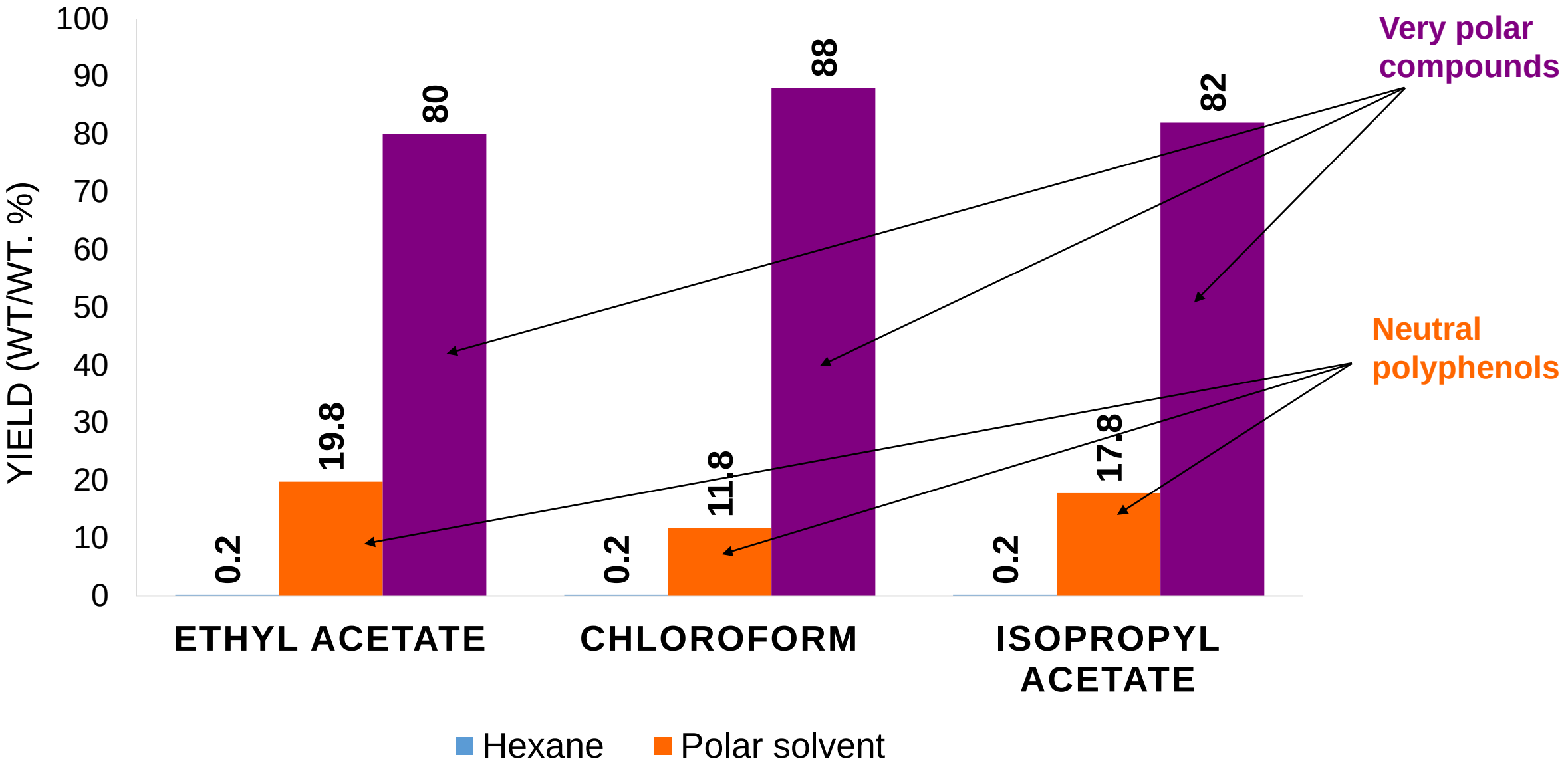
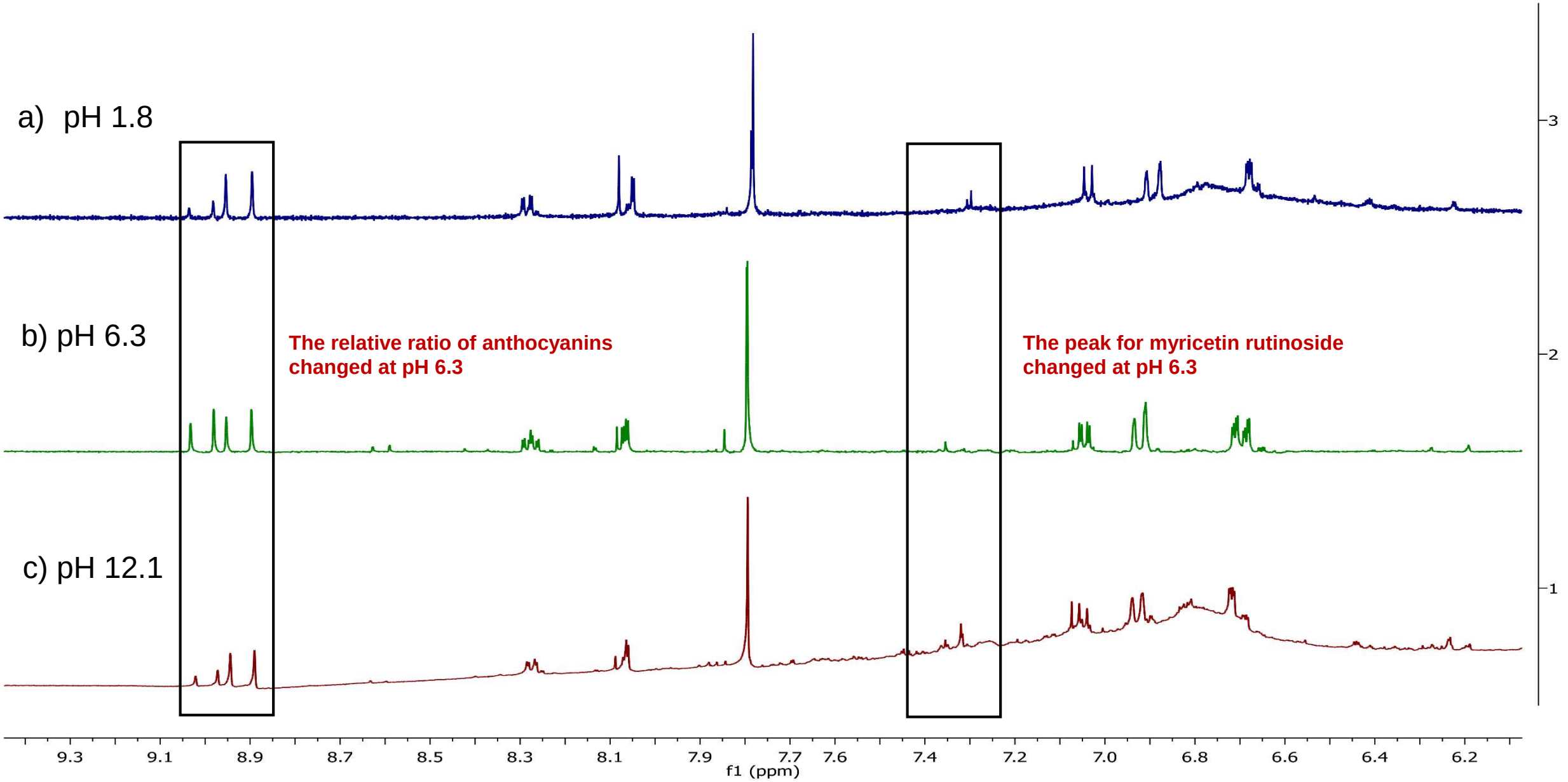
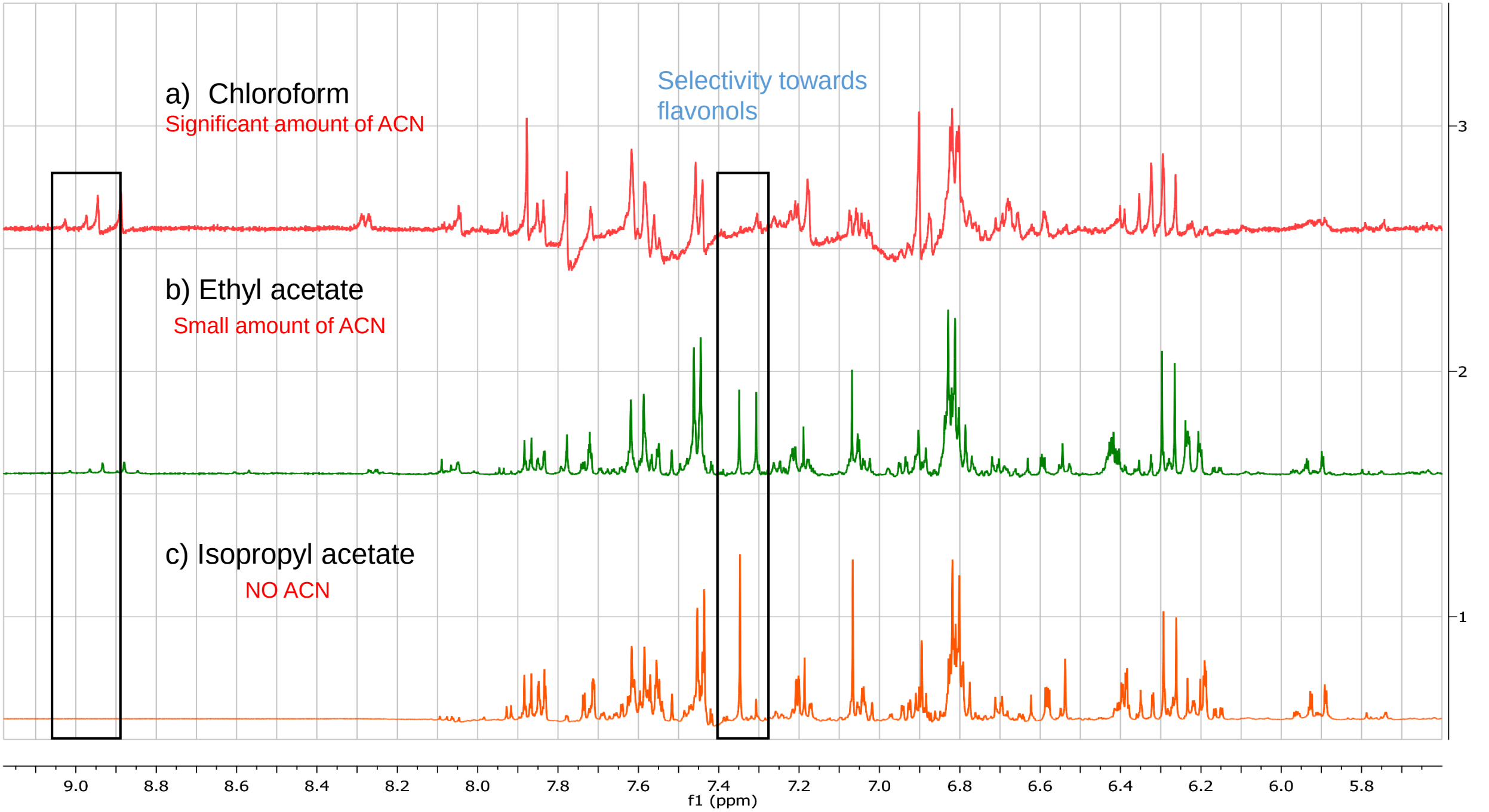
ADDITIONAL SLIDES

Selected solvents: hexane, ethyl acetate, ⁱpropyl acetate and chloroform partitioned against aqueous extract (pH 1.8; HCl)



^{1}H NMR for the Aqueous Layers (in MeOD-TFA 95:5) after the pH-controlled partitioning experiments




a) Chloroform
Significant amount of ACN
Selectivity towards flavonols
b) Ethyl acetate
Small amount of ACN
c) Isopropyl acetate
NO ACN
3
2
1
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
f1 (ppm)