Sample preparation and chromatographic techniques in NADES extract analysis

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Before starting…….

- Why NADES?
- It is a different, novel option to evaluate.
- It has proved to increase solubility of “difficult” molecules.
- It does not have to be removed before use if made with use-compatible ingredients.
- Most ingredients are GRAS compliant

Not an alternative….. a welcome option

Before starting…….

Still to be done: evaluate “greeness”…

Among others:

- LCA (life-cycle assessment): many individual ingredients have been assessed but not the NADES themselves.
- Can they be recycled: evaluate each application to see how they rate in a circular economy framework.

General approach for extract analysis

Qualitative information:

- Is this extract really authentic, i.e., is it made with the correct species, part and extraction solvent, method, etc.?

Quantitative information:

- Was the extract made with the correct ratio of material: solvent?
- Was it made with quality starting materials?

Qualitative information

Chemical profile

- Generally analyse the chromatographic profile such as TLC, HPLC
- Look for coincidence of the whole profile, especially ratii of some components.
- Presence of markers of material but also absence of markers (negative markers) of adulterants.

NADES extracts….
Potential food /cosmetic ingredients?

- Removal of extraction solvent not required
- Increased stability of components
- Possible added value from extraction solvent component, e.g. xylitol
- Concentrated source of active components
- GRAS ingredients
- Liquid easily combined into liquid / semi- solid formulations.

Organic/aqueous solvent extract - NADES extract

Conventional	NADES
Low viscosity	Medium to high viscosity
Low M*r* (50-100 Da range)	Relatively higher (100-360 Da)
Non-ionisable	Generally ionisable
Volatile	Non-volatile
Thermally stable	Generally thermally unstable
No acid/base character	Often acid/bases

Analytical methods

Spectroscopic:

- UV/Vis
- MS
- ^{1}H NMR
- IR

Chromatographic:

- HPLC/DAD
- HPLC/ELSD/RI
- LCMS
- GCMS

Sample preparation: 3 approaches

- Dilution
- Extraction
- SPE

-Sample pre-treatment (I): Dilution/injection

Applicable if:

- Samples require no pre-concentration
- No solvent incompatibility with the analytical method.

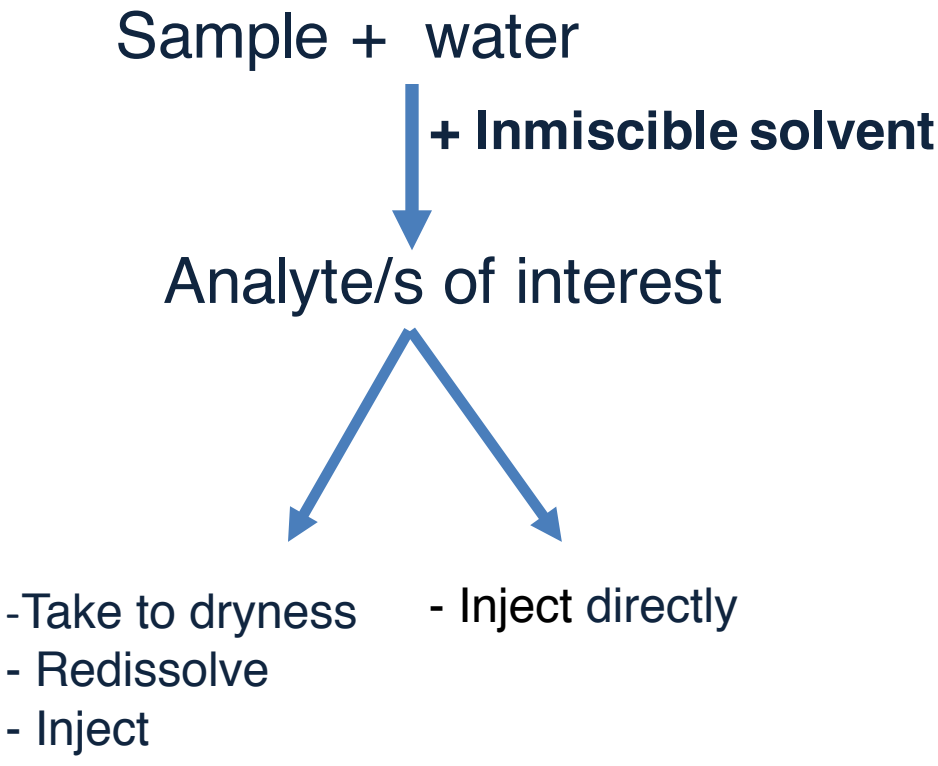
HPLC/DAD; UV/Vis

How is it done?

- Simple dilution with water or mobile phase (preferable)
- Filtration with membrane filter (0.2 μ).

Sample pre-treatment (II)

Liquid-liquid extraction



Sample pre-treatment (III)

Solid Phase extraction (SPE)

- Dilution
- Extraction on C18 (sample retention)

or

IEX (solvent retention for ionisable NADES)

Example: Calendula

Calendula NADES extract

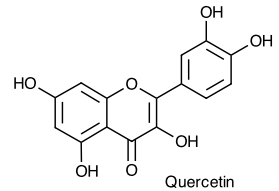
(xylitol: citric acid: water) or (glucose : citric acid : water).

- Carotenoids: lutein, α- and β- carotene, etc
- Flavonoid glycosides: rutin, quercetrin, etc
- Flavonoid aglycones: quercetin, isorhamnetin, etc.

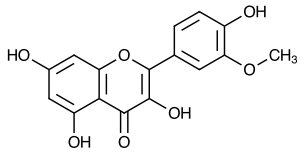
Calendula: chemical composition (I)

- Flavonoid aglycones
- Flavonoid glycosides

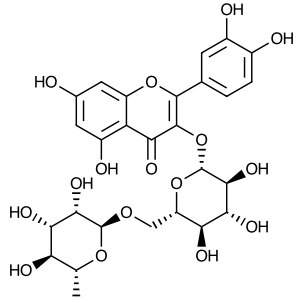
Hydrophilic, soluble in methanol/water; methanol; ethyl acetate; butanol, etc.

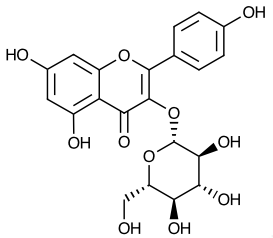


Quercetin

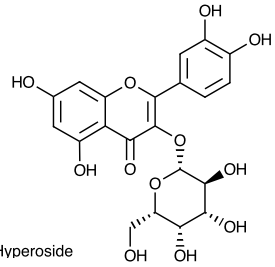


Isorhamnetin

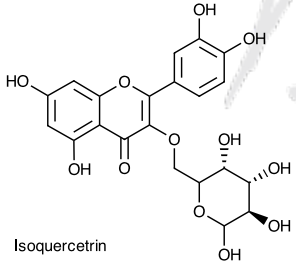




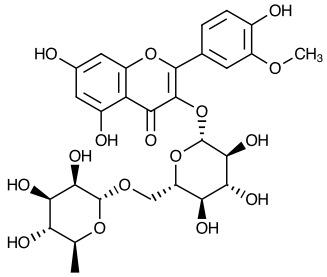
Astragalin



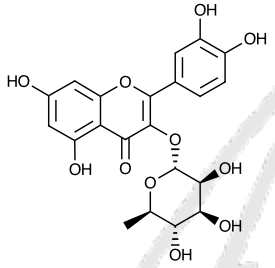
Hyperoside



Isoquercetrin



Narcisina

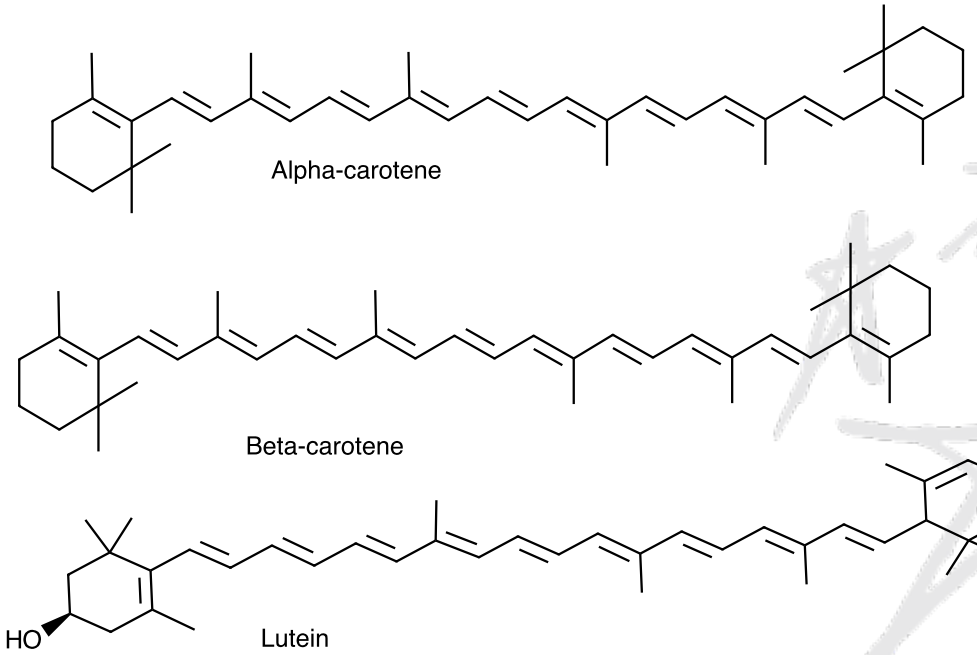


Quercetrin

Calendula: chemical composition (II)

Carotenoids:

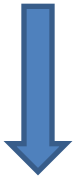
Lipophilic, soluble in non-polar solvents CH_2Cl_2, toluene, hexane.



Note: in this case, water content is critical: even starting with fresh material instead of dried material reduced the yield dramatically

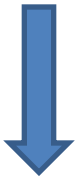
Strategy for flavonoids

(i) HPLC/DAD ; UV/Vis



Direct dilution with water

(ii) LCMS; NMR



(i) Ethyl acetate
(ii) Take to dryness
(iii) Redissolve in mobile phase/ NMR solvent

Strategy for carotenoids

For all analytical platforms:

(i) Dilute with H_2O

(ii) Extract with CH_2Cl_2

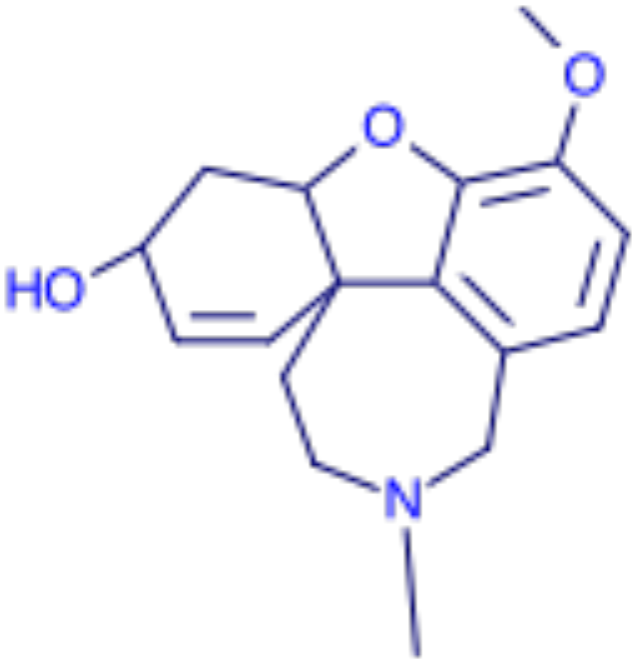
(iii) Dilute with mobile phase if pre-concentration not required

(iv) HPLC: C18 / ACN: H_2O – ethyl acetate: MeOH

or

(v) Total carotenoid content (UV/Vis)

Example: Alkaloids in Narcissus bulb



Galanthamine

Extraction/analysis flow-chart

200 mg liophyllized bulb material
+
NADES components (solid)
+
Sand

50° C (60 min) Pre-heating

2 x extraction cycles
50°/50 bar
2 min

Discharge of NADES extract (5 min)

GCMS
Column: DB5 (30mx0.25mmx 0.25µ)

HPLC/DAD
Column: Gemini NX-C18 3.0µ C18, 100 x 4.6 mm
Mobile phase: Methanol:water:TEA (50:50:0.3)
Detection: 279 nm
Flow: 0.7 ml/min

GC/MS
Pre-treatment:
Basic extraction (NaOH→Cl_3CH)
Take to dryness
Redissolve with MeOH
Derivatise

HPLC/DAD
Pre-treatment:
Sample+MeOH (2 h)
Dilution with mobile/phase
Centrifuge
Adjust pH≈11
Filter with PVDF/RC 0.2µ

NADES alkaloid extract

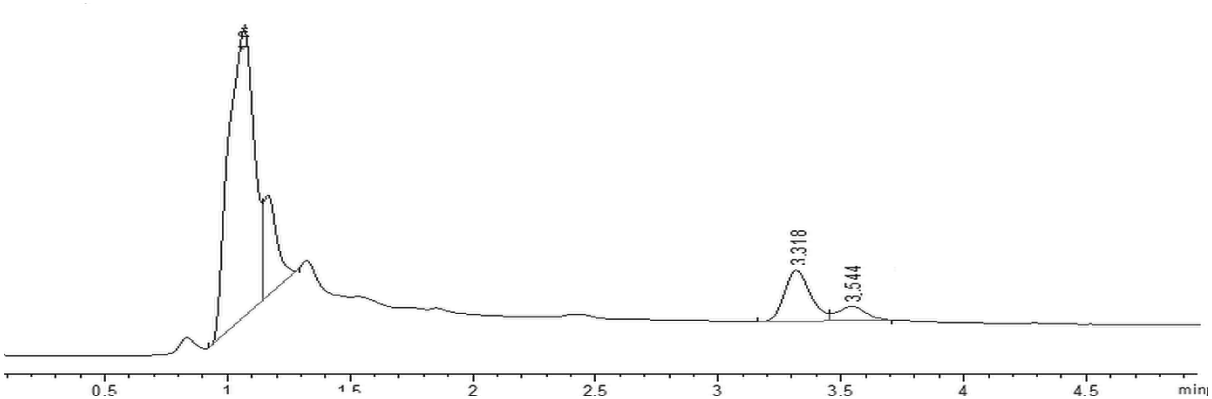
Issues to consider:

Can a NADES component interact with analytes?

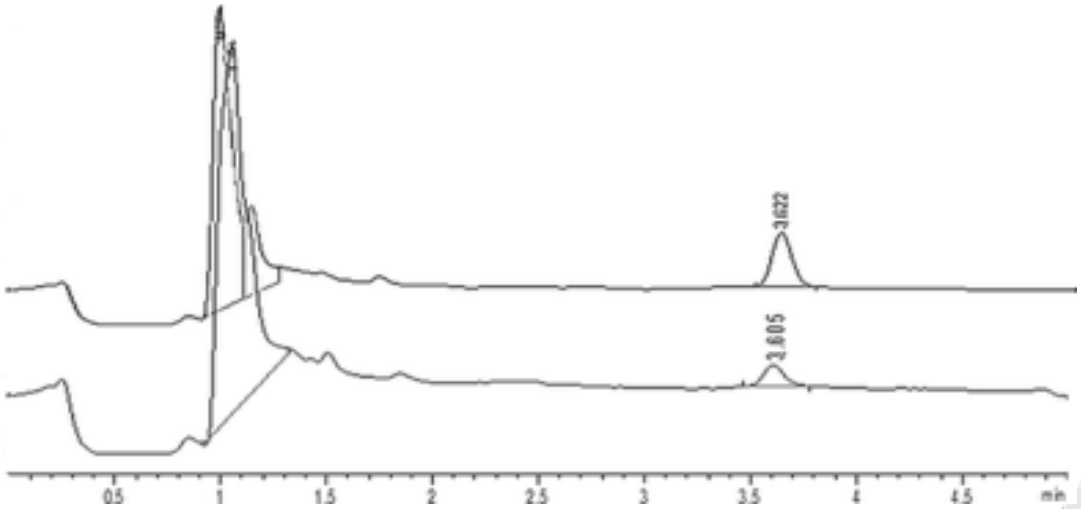
Weak acids, such as citric acid / malic acid interact with some alkaloids forming complexes.

NADES extract of Narcissus (malic acid: glucose: water)

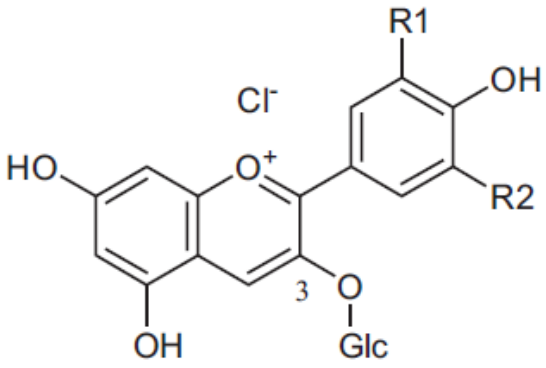
pH<10



pH>10

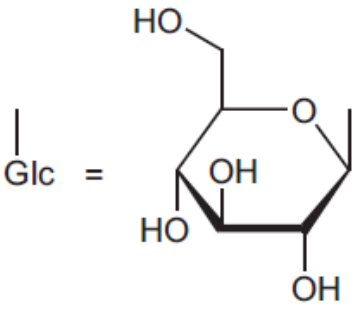


Example:Blueberry/ bilberry extract Anthocyanins



Cyanidin R1 = OH R2 = H
Delphinidin R1 = R2 = OH
Malvidin R1 = R2 = OCH_3
Peonidin R1 = OCH_3 R2 = H
Petunidin R1 = OH R2 = OCH_3

3-monoglucosides



β-D-glucopyranosyl

HPLC/DAD- UV/Vis

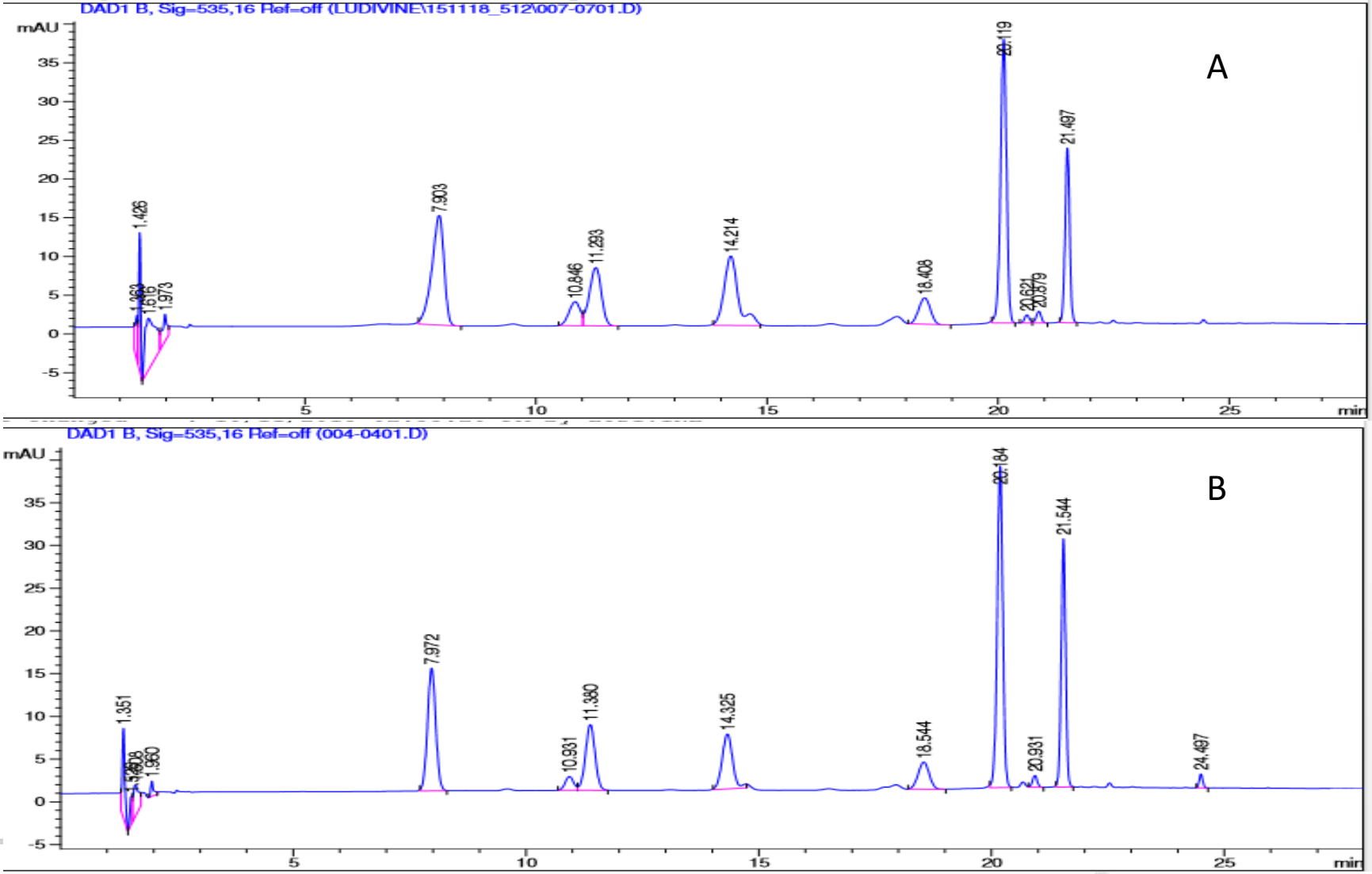
(xylitol : citric acid: water); (glucose: citric acid : water)

Possible with direct dilution or SPE

- Cannot be extracted with any immiscible organic solvent.

- Salting out/immiscible organic does not work.

NADES blueberry (A)
(EtOH:H_2O: HCl) blueberry (B)



LCMS with direct dilution?

- Possible and easy when analytes have higher Rt's than NADES components:
- NADES components: usually unretained on C18 column.
- Divert the flow for first minute with a switching valve

Concluding....

Sample prep and analysis not a problem…but special consideration should be given to:

- **Extraction power** of hydrophilic polymers or large molecules with NADES is greater than usual solvents or even water.
  Example: **lectins** in bulbs caused build-up of pressure in HPLC column (had to be removed).
  Pectins are well extracted…not always welcome though!!

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Thank you!!!

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