Chemical profiling of natural extracts obtained with NADES systems using the CARAMEL approach

Hypericum perforatum in the neutral NADES Glycerol/H_2O/Fructose as a case study

Natural deep eutectic solvents (NADES) are green solvent systems, prepared by mixing hydrogen bond acceptors & donors in specific molar ratios (usually organic acids, sugars, polyols, quaternary ammonium salts or amino acids).

NADES are less toxic than classical organic solvents, non-volatile, non-flammable, and easily prepared. These intrinsic properties make them environmentally friendly and perfect systems to solubilize a wide range of molecules. Therefore, NADES used as extractants have become a focus of interest in the development of cosmetic, pharmaceutical, and food ingredients.

Analytical challenge

Chemical profiling of NADES extracts is often hampered by the low vapor pressure and high viscosity of the NADES matrix, which is very difficult to eliminate for analytical purposes. The metabolites of interest remain highly diluted and trapped in the NADES systems.

⇒ Accurate chemical profiling of NADES extracts can be performed by CARAMEL:

1 EXTRACT PREPARATION

Hypericum perforatum

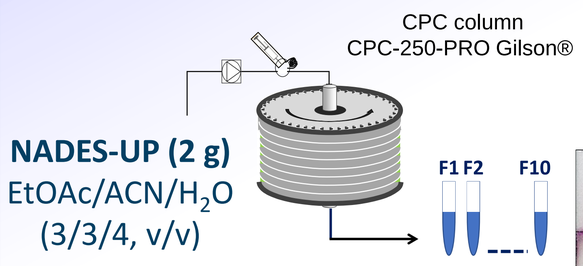
Aerial parts extracted with the **neutral NADES Glycerol/H_2O/fructose (1/1/3, w/w)**

2 LIQUID-LIQUID EXTRACTION in the biphasic system EtOAc/ACN/H_2O (3/3/4, v/v)

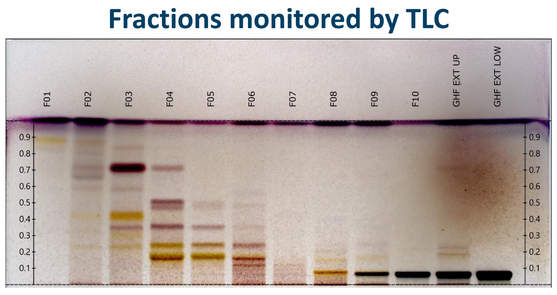
NADES extract

- NADES-UP — **5.4% in mass** but high chemical diversity
- NADES-LOW — **94.6% in mass** glycerol and fructose from the NADES + hydrophilic constituents (mainly sugars)

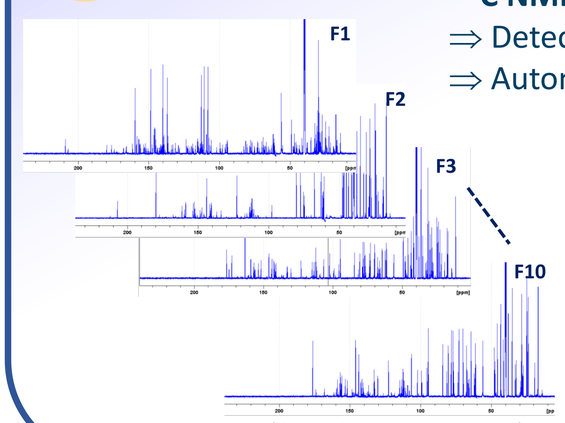
3 CENTRIFUGAL PARTITION CHROMATOGRAPHY ⇒ Fraction series from NADES-UP



Fractions monitored by TLC



4 NMR ANALYSIS OF ALL CPC FRACTIONS



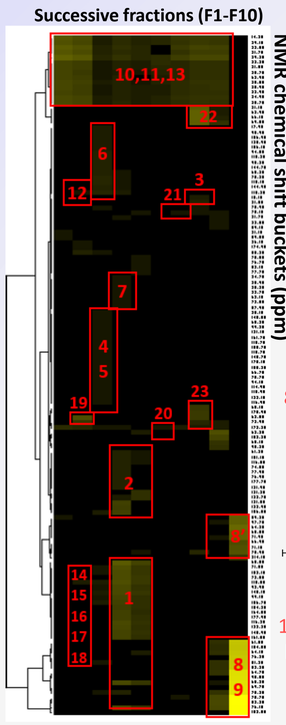
(Bruker 600 MHz, cryoprobe)

^{13}C NMR spectra
⇒ Detection of all metabolite carbon positions
⇒ Automatic peak picking and bucketing

ppm	f1	f2	f3	...	f15
16.3	2E+08	1E+08	1E+08	...	0
17.5	0	0	4E+07	...	0
18.7	1E+08	1E+08	9E+07	...	0
⋮	⋮	NMR signal intensities			⋮
177.7	0	0	0	...	4E+07
178.1	6E+07	6E+07	5E+07	...	0
199.5	7E+07	5E+07	3E+07	...	0

5 2D MAPPING OF ^{13}C NMR DATA by Hierarchical Clustering Analysis

⇒ Mesure NMR signal similarities caused by the same compounds eluted in several successive fractions
⇒ Each ^{13}C NMR cluster represents a carbon skeleton



METABOLITE IDENTIFICATION

1: Hyperoside
2: Hirsutrin
3: Rutoside
4: (-)-Epicatechin
5: (+)-Catechin
6: Quercetin 3-*O*-rhamnoside
7: 3-Hydroxy-4,4-dimethyl-γ-butyrolactone
8: β-D + α-D-glucose + α-D-fructopyranose
9: Saccharose
10: Oleic acid
11: Linoleic acid
12: Compound X (New compound)
β-D + α-D-fructose + β-D-fructopyranose
13: Triacylglycerols R1,R2,R3 = fatty acids
14: *p*-Hydroxy benzoic acid
15: Benzoic acid
16: Loliolide
17: Syringol
18: Vanillic acid
19: Glycerol (solvent)
20: Protocatechuic acid
21: Phenylethyl -glucoside
22: 1-Monoacetin (Solvent)
23: 2-Monoacetin (Solvent)

⇒ NMR database of natural molecules
⇒ Chemical structures fully validated by interpretation of 2D NMR data

⇒ A diversity of metabolites, mainly quercetin glycosides, catechin, and phenolic acids, were unambiguously identified in the *Hypericum perforatum* extract obtained with the NADES glycerol/H_2O/fructose (1/1/3, v/v).

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