

CRETUS Matrix solid-phase dispersion-high-performance liquid chromatography with diode array detection as a sustainable alternative for carotenoids analysis in green and brown macroalgae

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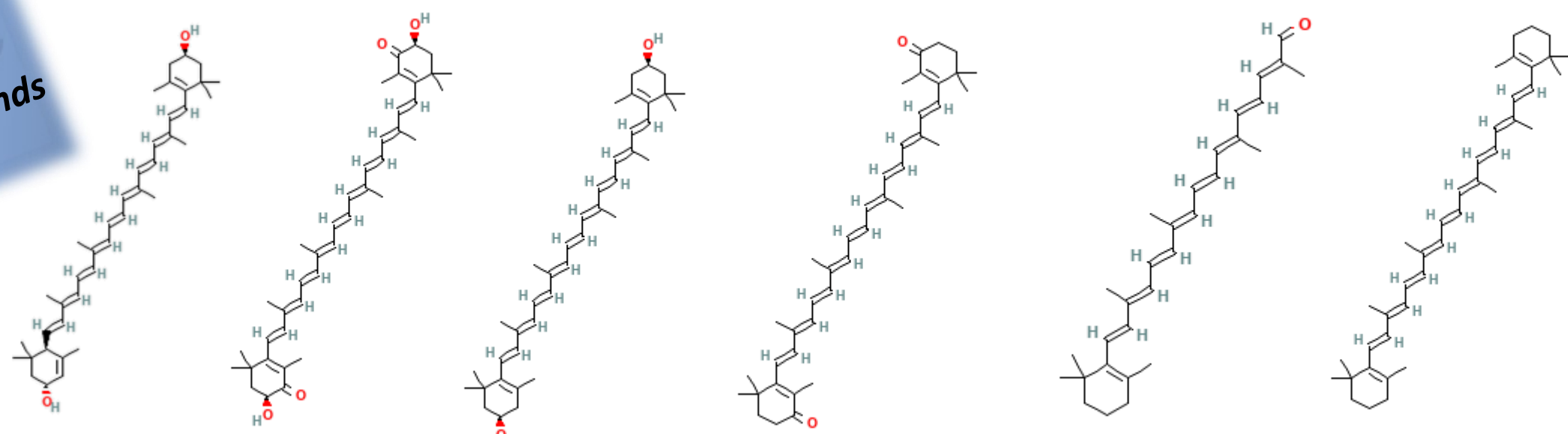
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INTRODUCTION & AIM

Carotenoids are lipophilic compounds with conjugated structures, making them susceptible to thermal degradation, especially when exposed to high temperatures for extended periods. The use of matrix solid-phase dispersion (MSPD), a green and low-cost ambient temperature and pressure technology, is a suitable alternative to classical procedures such as Ultrasound-Assisted Extraction (UAE), Pressurized Liquid Extraction (PLE), or supercritical CO₂ extraction.

The aim of this work is the optimization of a MSPD followed by High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) methodology to simultaneously determine carotenoids in macroalgae. The MSPD experimental parameters affecting extraction, dispersant, sample/dispersant ratio, and extraction solvent were optimized by experimental design to obtain the highest extraction efficiency.

Target compounds



Samples



GREEN ALGAE
Codium fragile



BROWN ALGAE
KOMBU (*Laminaria ochroleuca*)

METHOD

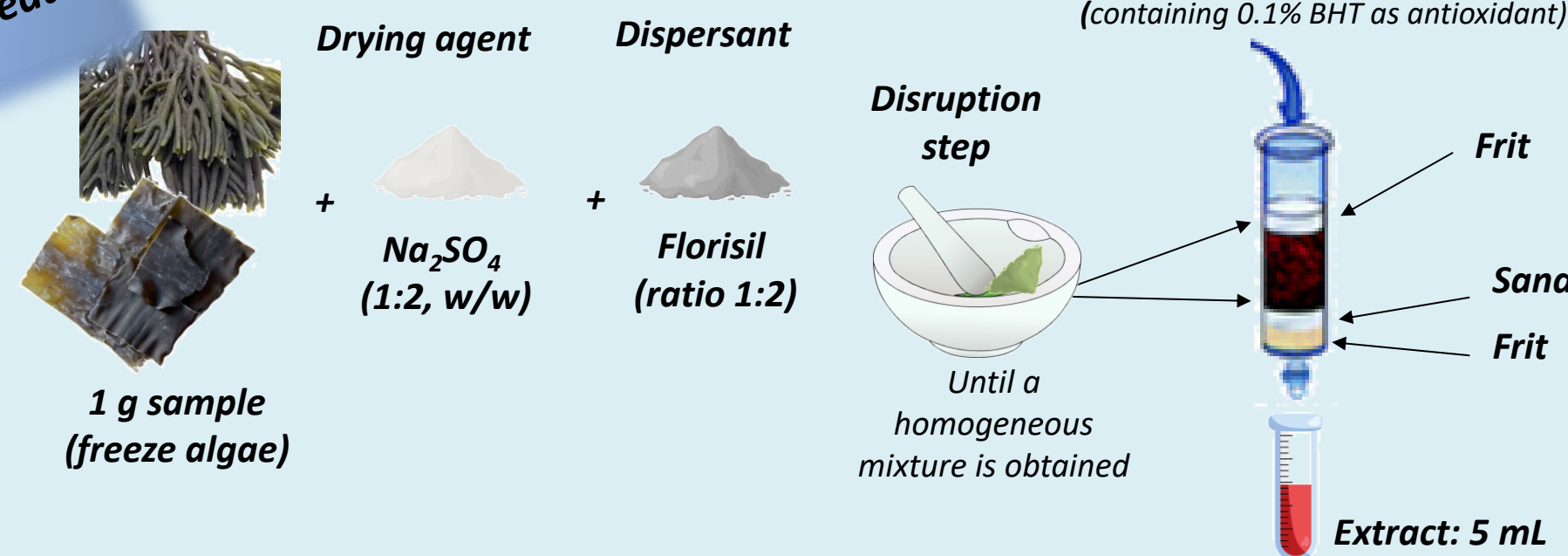
MSPD optimization was carried out employing non-spiked algae sample. The following experimental parameters were optimized by a factorial design.

Parameter	Factors			
Dispersant	Sand		Florisil	
Sample-dispersant ratio (w/w)	1:2		1:4	
Elution solvent	Acetone	Methanol	Methanol/acetone	Methyltertbutylether

16 experiments

MSPD procedure

MSPD procedure under the optimized experimental conditions



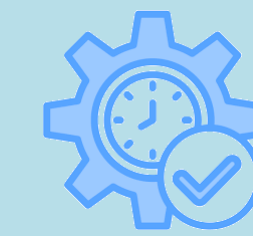
HPLC-DAD analysis

HPLC-DAD optimized conditions

Mobile phases composition (%) & gradient

Time (min)	A	C	D
10	20	5	75
15	50	0	50
20	80	0	20
25	90	0	20
30	90	0	20
35	20	5	75

Column
C30 (5 µm, 4.6 x 250 mm)



Total run time
35 min



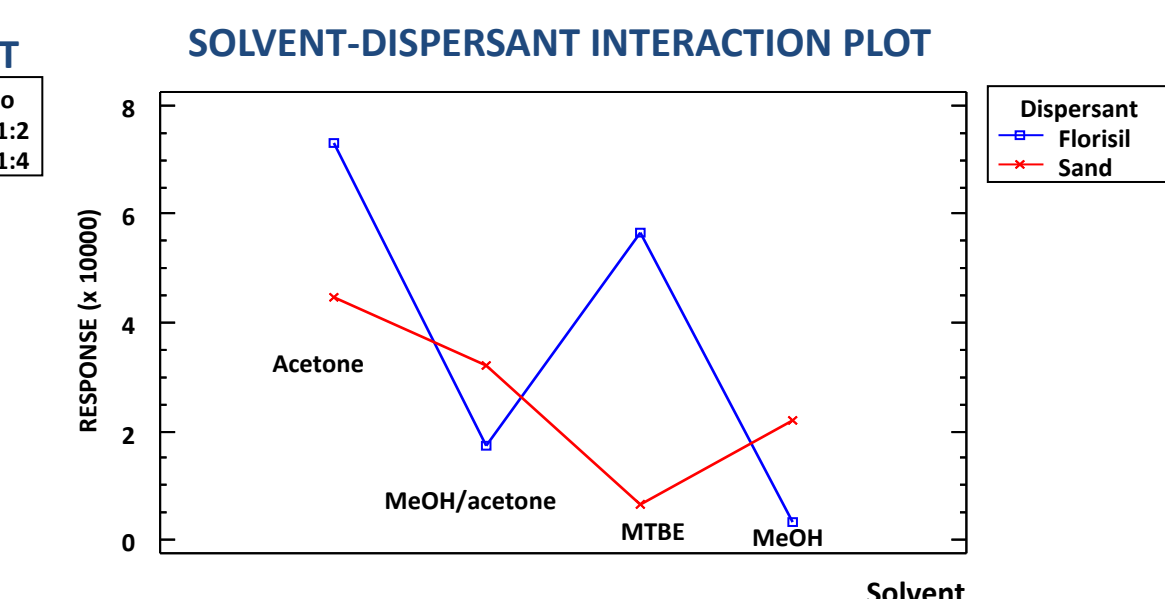
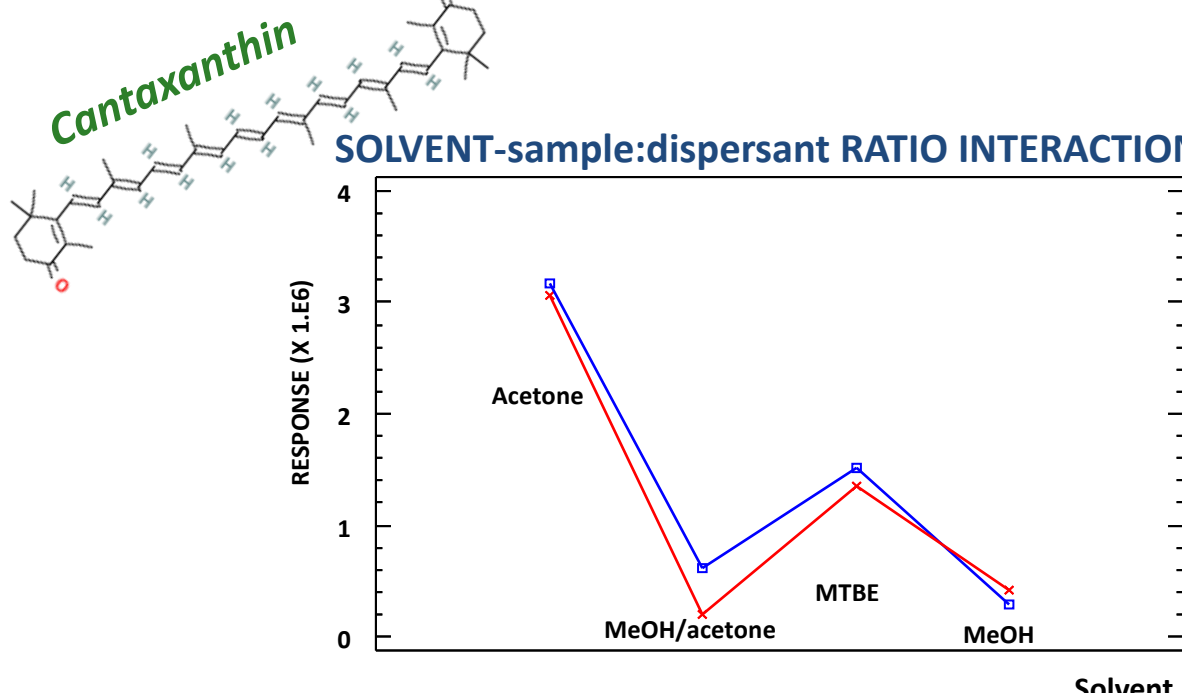
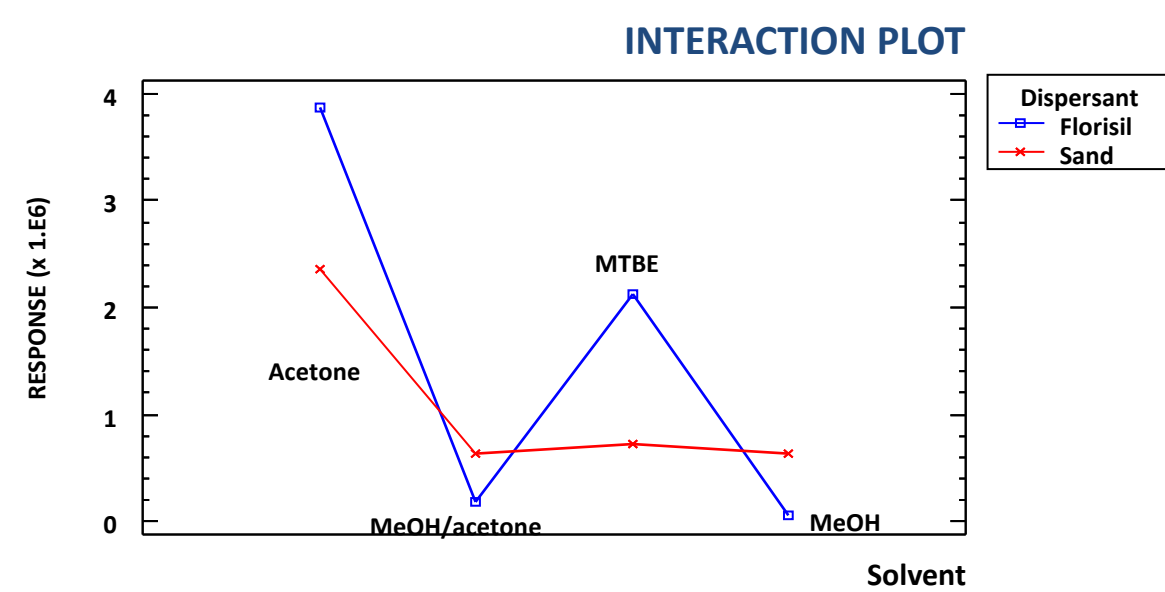
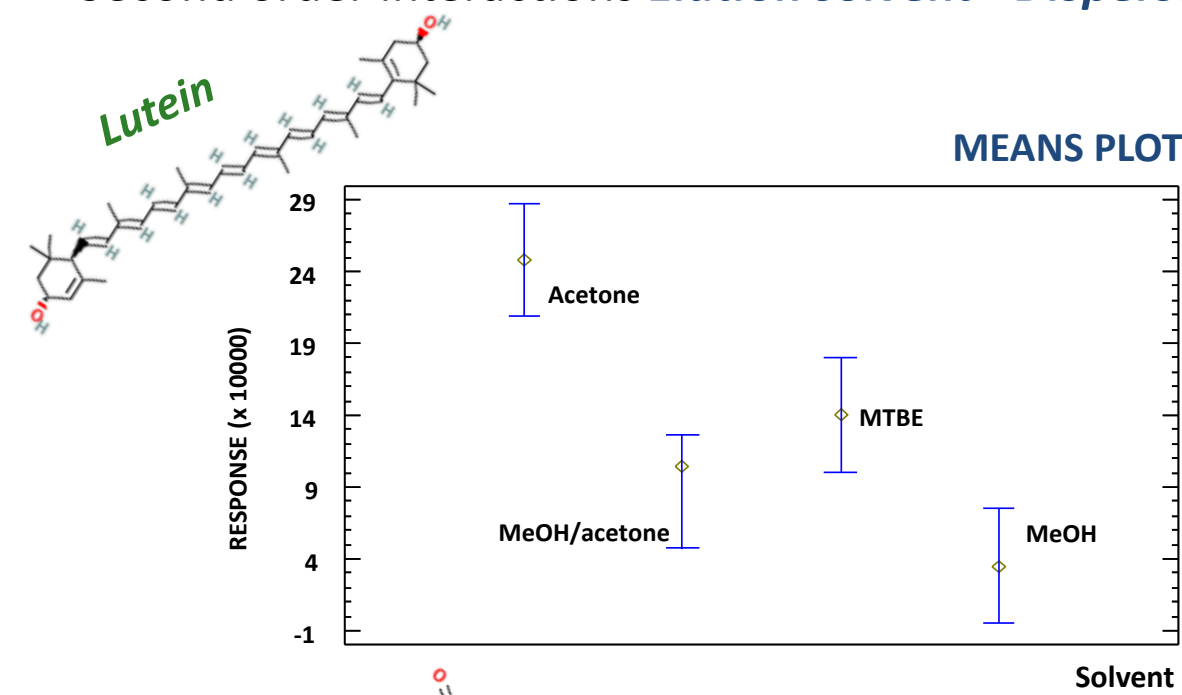
Oven temperature
15 °C



Injection volume
10 µL

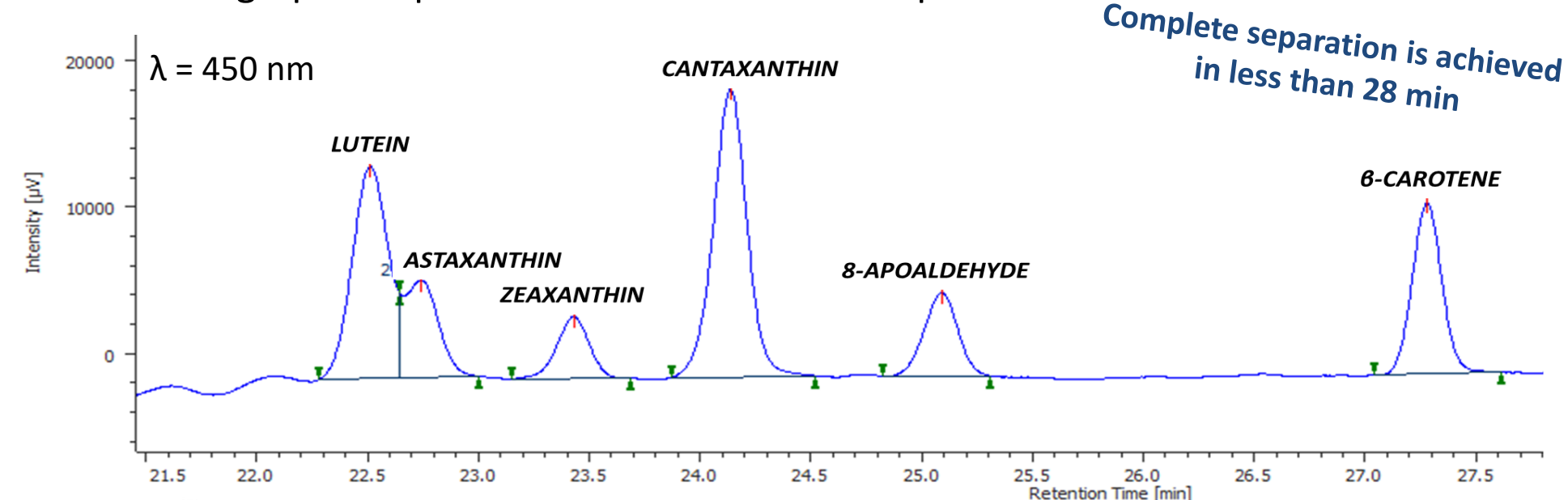
RESULTS & DISCUSSION

- The **elution solvent** was statistically significant (p-value < 0.05) for all target carotenoids.
- Second order interactions **Elution solvent - Dispersant** were statistically significant for lutein and cantaxanthin



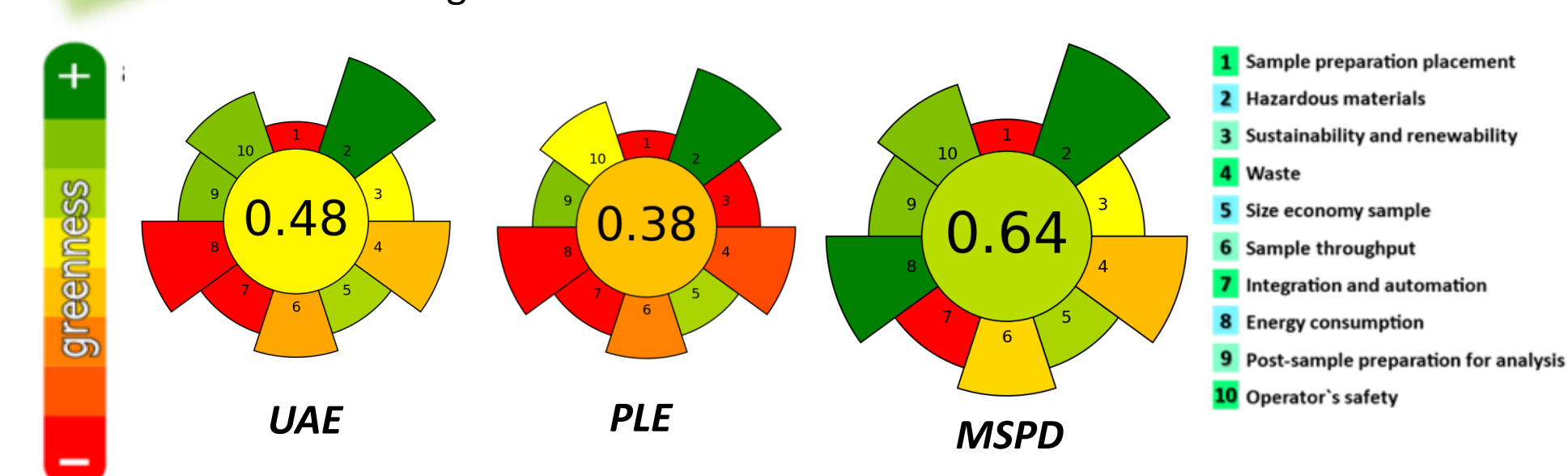
Optimized MSPD experimental conditions involve: **FLORISIL** as DISPERSANT
1:2 (w/w) RATIO sample-dispersant
ACETONE as ELUTION SOLVENT

- HPLC-DAD instrumental conditions were optimized to achieve the best chromatographic separation and carotenoids response



Greenness assessment

- The greenness of MSPD was assessed through AGREEprep software [1].
- MSPD has been compared with classical techniques (UAE and PLE), usually employed to extract carotenoids from complex matrices such as macroalgae



- ✓ MSPD operates at ambient temperature and pressure, using low-cost and reusable materials, making it a green and sustainable alternative for extracting carotenoids from macroalgae.

REFERENCES & ACKNOWLEDGEMENTS

[1] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis. AGREEprep – Analytical greenness metric for sample preparation, *TrAC – Trend. Anal. Chem.*, 149 (2022), 116553.
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