Systematic Fractionation Using Complementary Developing Solvent Technique in Flash Chromatography: A Key Step in Dereplication Strategies for Natural Products

Jason Fauquet[1], Adam Ellatiff[1], Claudio Palmieri[1], Pierre Duez[1], Bertrand Blankert[2], Mathilde Wells[2] and Amandine Nachtergael[1]

[1]Laboratory of Therapeutic Chemistry and Pharmacognosy, Research Institute for Health Sciences and Technology, Research Institute for Bioscience, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons (UMONS), Belgium

[2]Laboratory of Pharmaceutical Analysis, Research Institute for Health Sciences and Technology, Research Institute for Bioscience, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons (UMONS), Belgium

What?

COMPLEMENTARY DEVELOPING SOLVENT (CDS)

A trio of 3 solvent mixtures: Low, Medium, and High-Polarity Developing Solvent (LPDS, MPDS, HPDS).

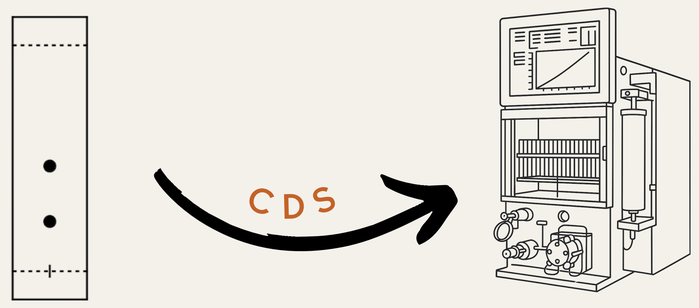
Mixture name	Solvents	Proportion (v/v)
HPDS	Ethanol, DCM, Water, FA	43:43:11:3
MPDS	MTBE, THF, Water, FA	61:36:1.5:1.5
LPDS	Toluene, Ethyl acetate	9:1

- Significantly enhance chromatographic resolution.
  - Covering a broad range of selectivities.
- Three complementary chromatographic fingerprints

Originally, developped for untargeted analysis in High-Performance Thin-Layer Chromatography (HPTLC).

OUR OBJECTIVE ?

Adapting CDS from HPTLC to Flash chromatography



How?

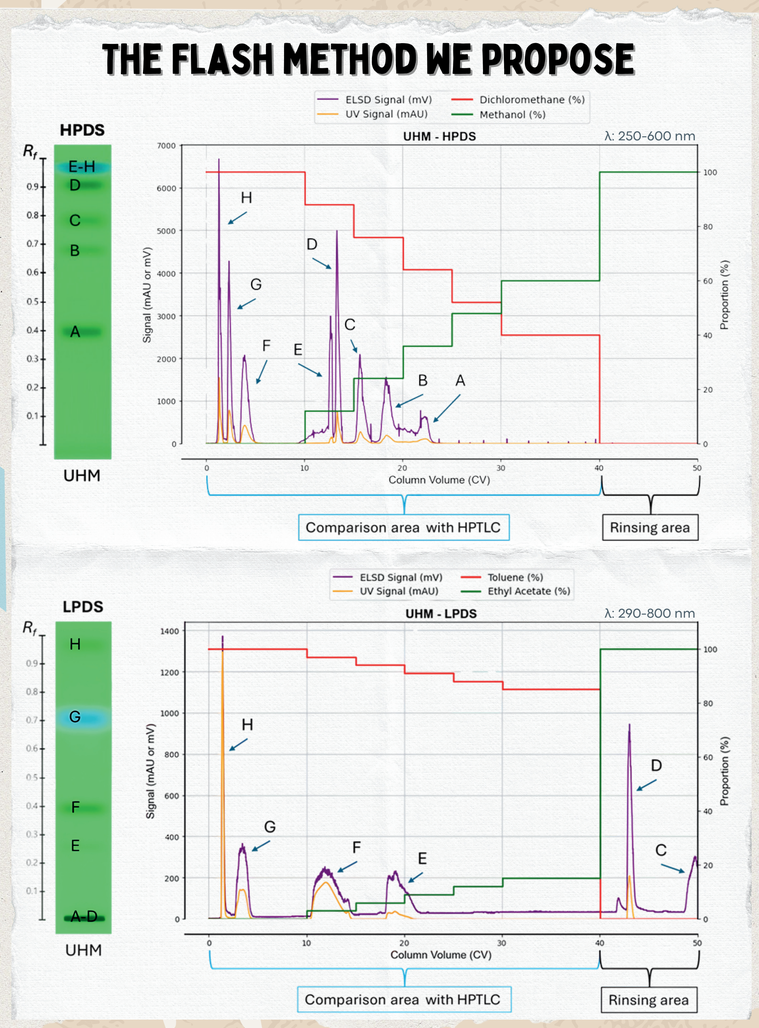
Using the same System suitability test (SST) covering a wide polarity range for direct comparisons :

Universal HPTLC Mix (UHM)

Label	Name	Log P	HPTLC concentration (mg/mL)	Flash concentration (mg/mL)
A	Guanosine	-1.90	0.5	1
B	Sulisobenzone	0.88*	1	1
C	Thymidine	-0.93	1	1
D	Paracetamol	0.46	1	1
D	Phthalimide	1.15	2	1
F	9-hydroxyfluorene	2.84*	1	1
G	Thioxanthen-9-one	3.90*	0.01	1
H	Octrizole	5.88*	1	1

* Computed values (DrugBank, PubChem or SciFinder)

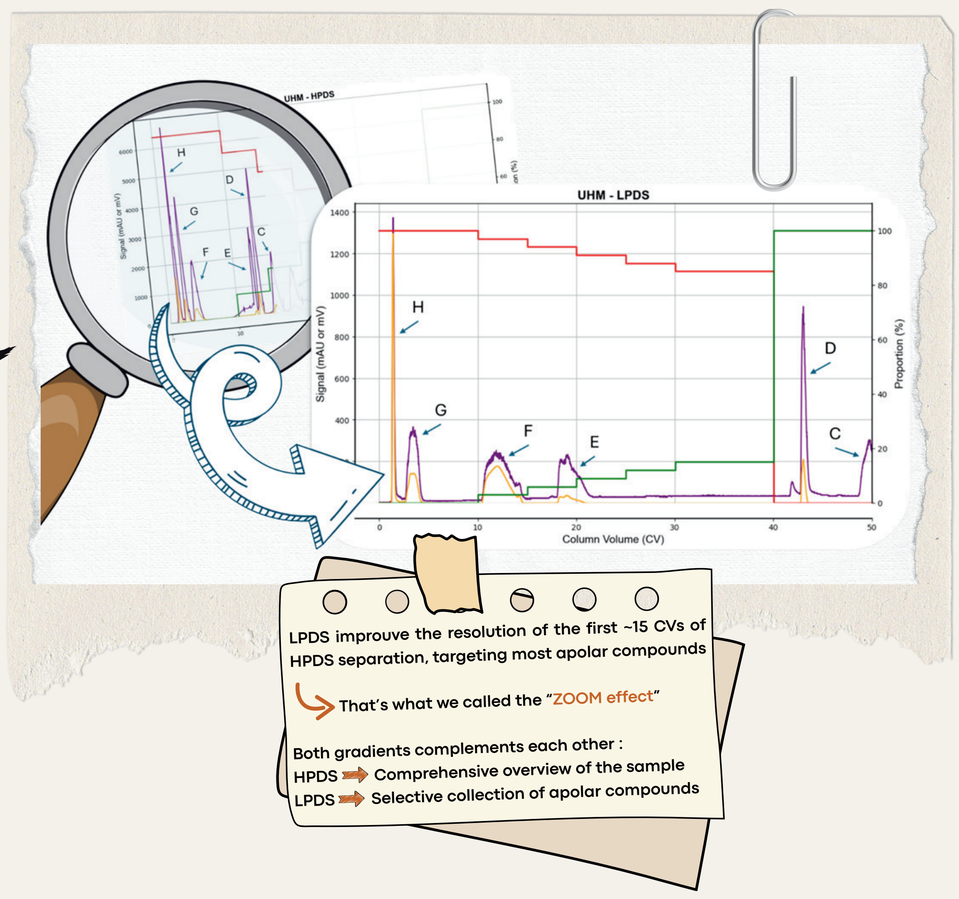
THE FLASH METHOD WE PROPOSE



Key features

1. Same elution order as for HPTLC & improved resolution.
2. UHM ensures reproducibility in solvent gradient control & maintains consistent elution patterns
3. The applicability of HPDS and LPDS gradients to diverse plant matrice allows systematization of untargeted studies

Note : Unfortunatly, MDPS was'nt succesfully develloped because of non-elution of compounds



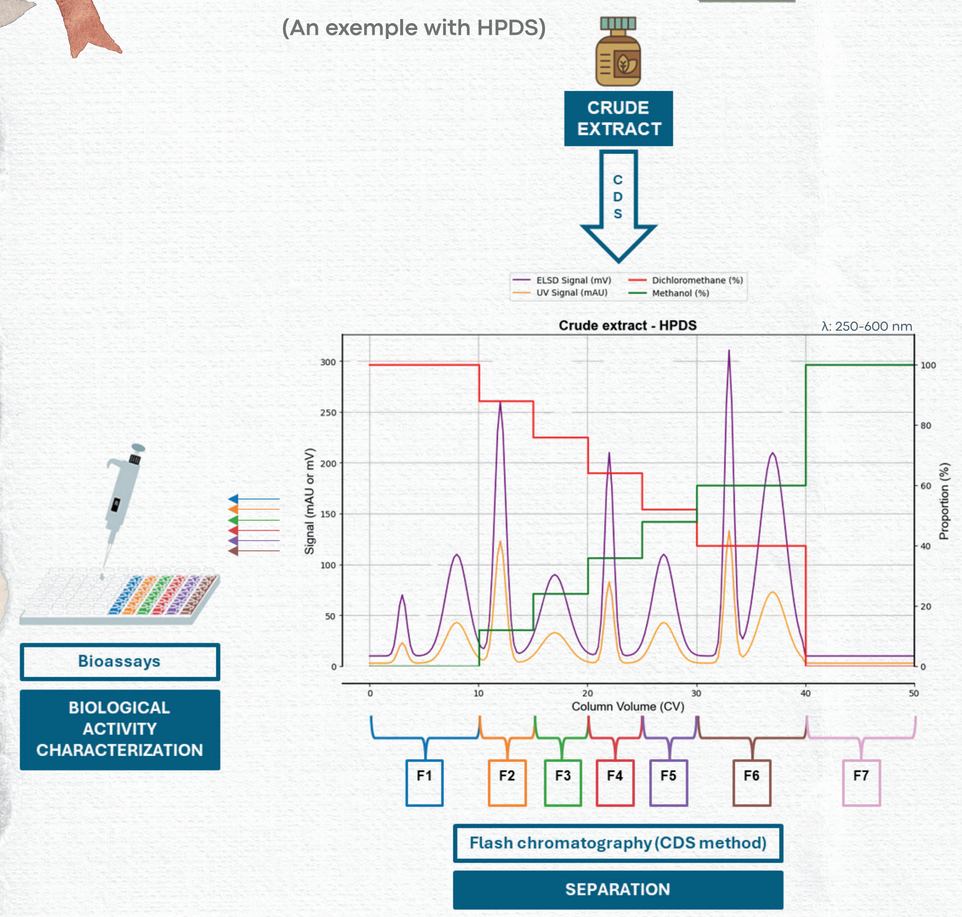
LPDS improuve the resolution of the first ~15 CVs of HPDS separation, targeting most apolar compounds

That's what we called the "ZOOM effect"

Both gradients complements each other :
HPDS ➡ Comprehensive overview of the sample
LPDS ➡ Selective collection of apolar compounds

THE WAY TO INCLUDE IT IN YOUR WORKLOW

(An exemple with HPDS)



BONUS

A new systematic fractionation strategy !

- Seven isocratic steps by gradients
- One fraction collected by isocratic step - F1 to F7
- Each fraction is enriched with compounds of similar polarity
- Reduced fraction numbers : 1 extract = max 14 fractions VS many more with peak-to-peak collection strategy
- Accelerates bioguided fractionation and untargeted metabolomics workflows

Contact me at : jason.fauquet@umons.ac.be

References

1. Do, T.K.T., et al., 2021. Journal of Chromatography A 1638, 461830.
2. Do, T.K.T. et al., 2022. JPC-J Planar Chromat 35, 299–311.
3. Do, T.K.T., Reich, E., 2023. JPC-J Planar Chromat 36, 317–325.

Aknowledgement

We express our sincere gratitude to Prof. R. Wattiez from the Proteomics and Microbiology (ProtMic) Unit for granting us access to the UMONS Bioprofiling Platform. The Bioprofiling platform used for proteomic analysis was supported by the European Regional Development Fund and the Walloon Region, Belgium.

Poster created with canva.com

Faculté de Médecine, Pharmacie et Sciences Biomédicales

health — UMONS RESEARCH INSTITUTE FOR HEALTH SCIENCES AND TECHNOLOGY

Therapeutic Chemistry Pharmacognosy CTP

NAPLES 2025

Service d'Analyse Pharmaceutique

UMONS Université de Mons