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Development of a miniaturized solid phase extraction of enzymatic oxylipins for a miniaturized analysis on a UHPLC-ESI-MS/MS Agilent

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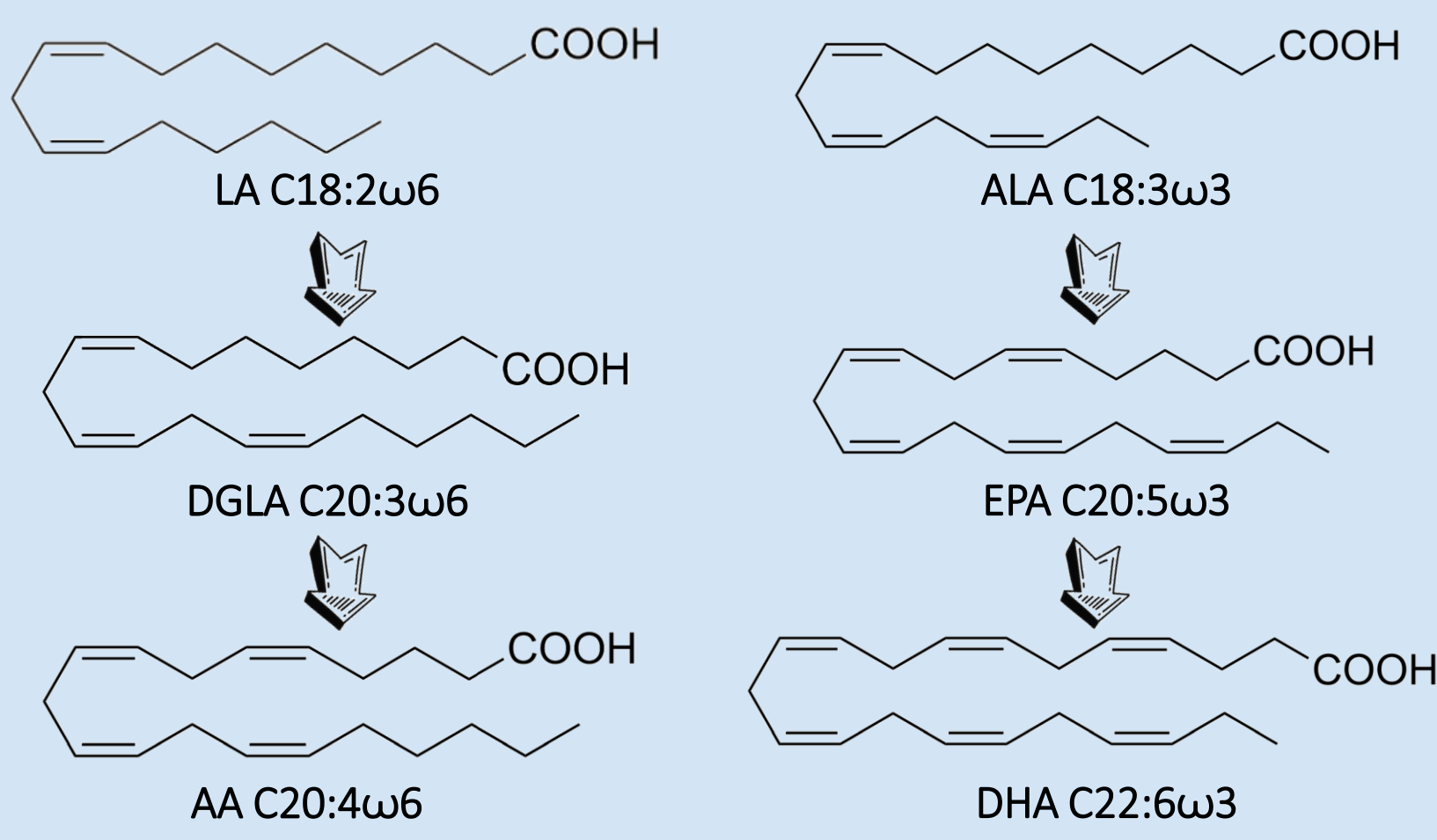
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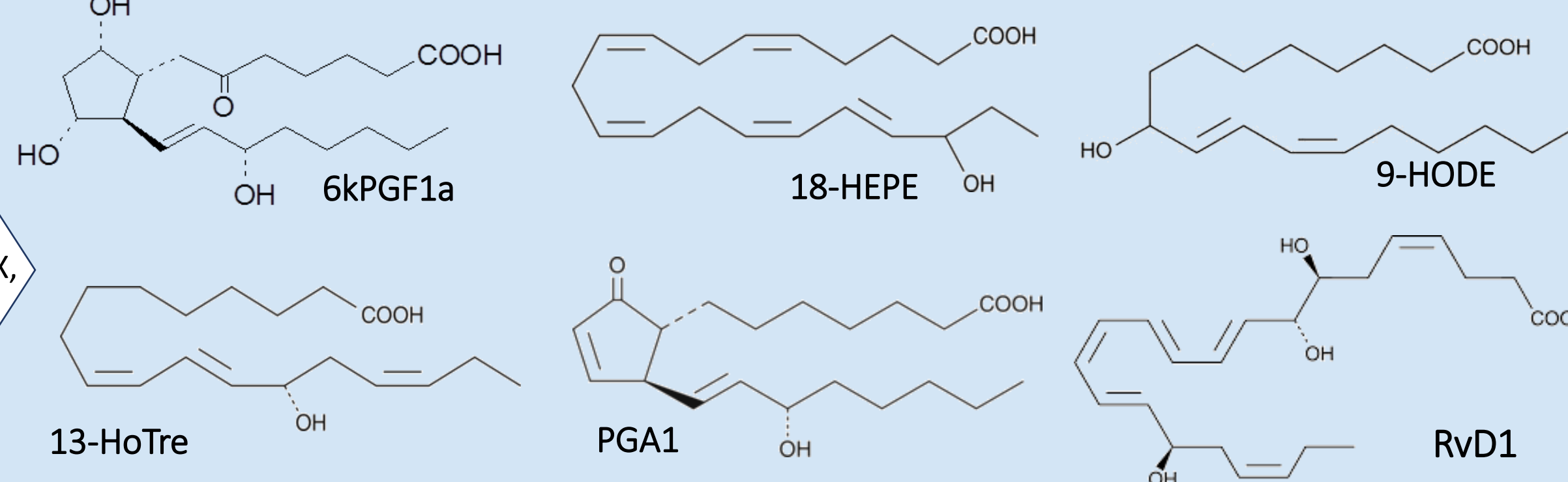
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From polyunsaturated fatty acids

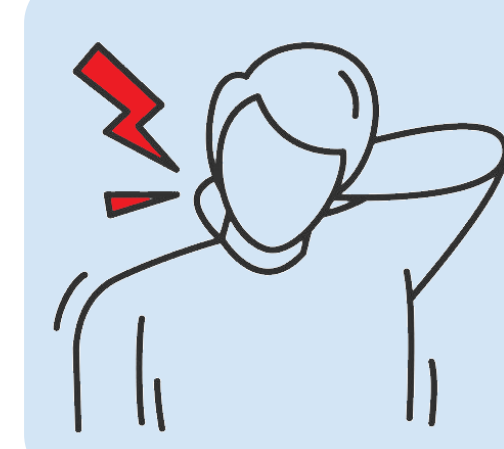


Generation of enzymatic oxylipins



Large diversity of structures Instables metabolites Very low concentration

Biological role



Promotion, inhibition and regulation of inflammation and its resolution

Aim of the work

Miniaturize the entire workflow of oxylipins analysis in order to gain in sensitivity by reducing sample and solvent volume

Sample Preparation

Protein precipitation

150 μ L Plasma / 260 μ L cold MeOH / 40 μ L ISTD **VS** 15 μ L Plasma / 20 μ L cold MeOH / 10 μ L ISTD

SPE, 30 mg

1 mL Conditioning: MeOH 1 mL Conditioning: H₂O/MeOH (90/10) 2 mL Diluted sample desposit 1 mL Washing: H₂O/MeOH (90/10) 1 mL Elution: Various

μ SPE, 2 mg

200 μ L 200 μ L 200 μ L 200 μ L 2x25 μ L

Eluate concentration Direct injection of the eluate

UHPLC-ESI-MS/MS

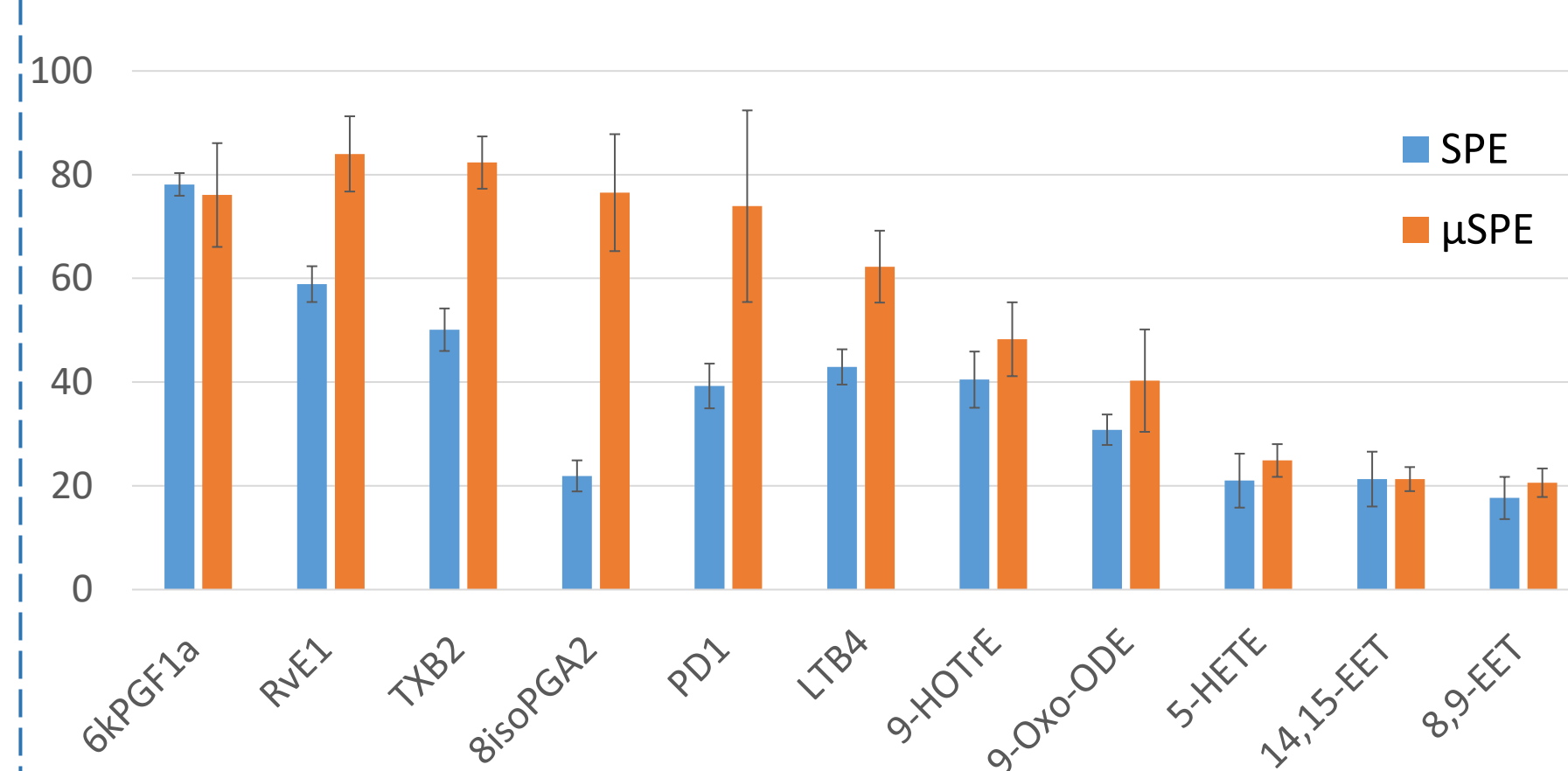


1290 Infinity - G6460 QqQ, Agilent

Column: Zorbax RRHD SB-C18, 100 x 2.1 mm, 1.8 μ m (Agilent) **ESI : Negative mode**
Solvent A: H₂O, 0.1% formic acid **Scan mode : DMRM**
Solvent B: ACN, 0.1% formic acid **56 transitions**
Flow rate: 400 μ L/min **20 internal standards**

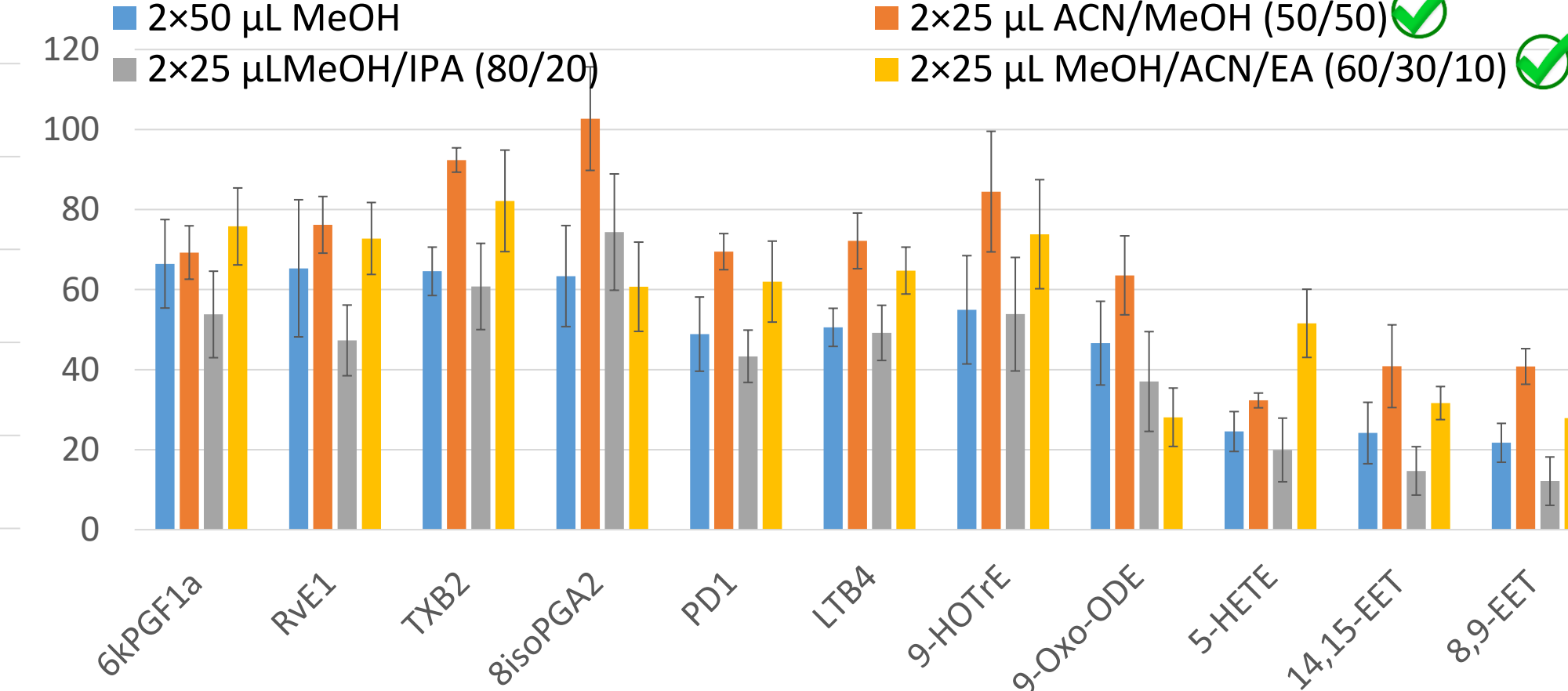
Results and discussion

1- Comparison of extraction recovery of enzymatic oxylipins on SPE and μ SPE: methanol elution



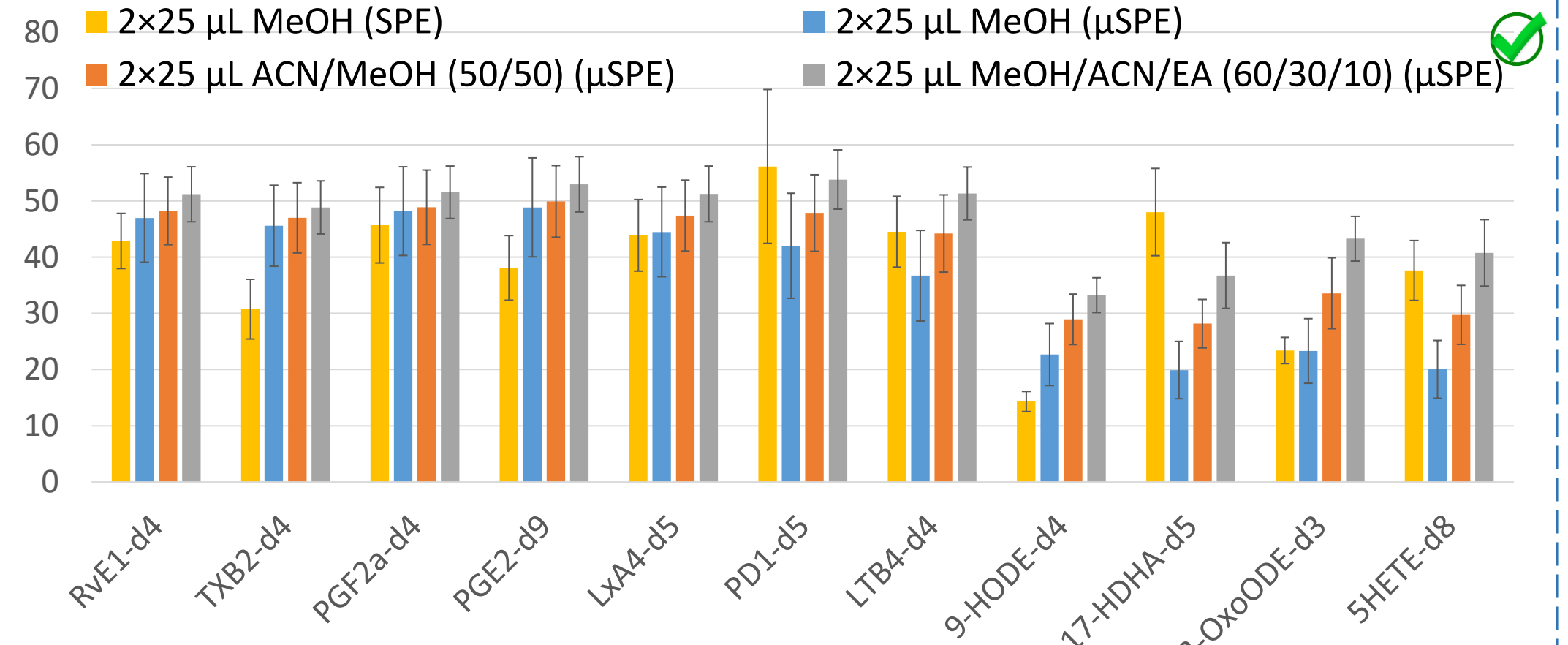
Higher recoveries obtained with μ SPE: ~80% for polar molecules and ~20% for apolar molecules

2- Optimization of the solvent elution on μ SPE plate - Recoveries (%) -



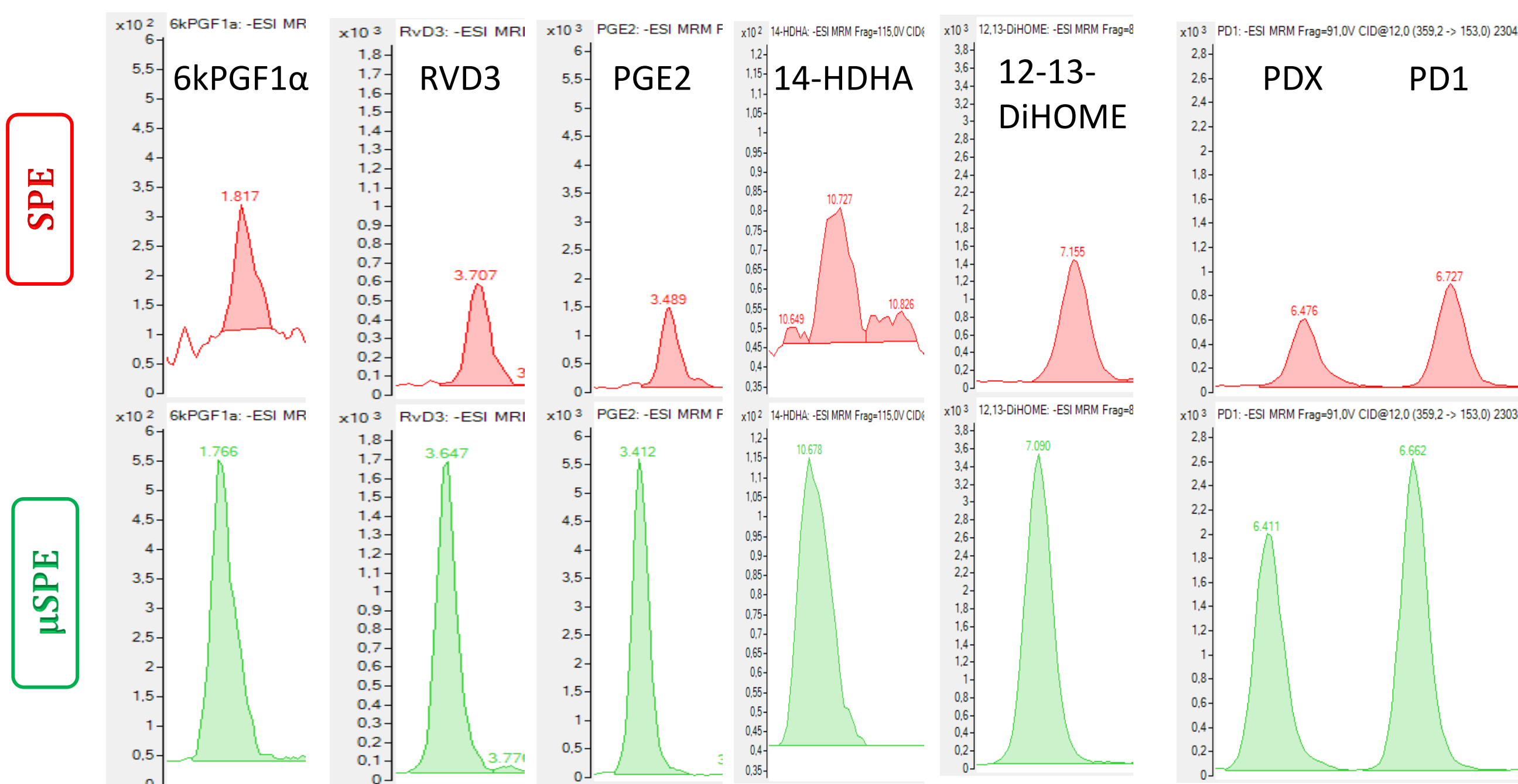
Higher recoveries for polar and apolar molecules obtained with 2x25 μ L MeOH/ACN (50/50) and 2x25 μ L MeOH/ACN/EA (60/30/10)

3- Extraction of plasma with best elution conditions on internal standards



Better recoveries for ISTDs obtained with 2x25 μ L MeOH/ACN/EA (60/30/10) on μ SPE plate

Comparison of EIC of different metabolites extracted by SPE vs μ SPE at 10pg/ μ L



→ Gain in sensitivity by μ SPE → Reduction of the signal to noise ratio in μ SPE

Next step : Analysis with a more sensitive analytical method UHPLC-ESI-MS/MS vs μ HPLC- μ ESI-MS/MS



1290 Infinity - G6460 QqQ, Agilent



Nexera X2 Mikros - LCMS 8060, Shimadzu

	Zorbax RRHD SB-C18, 100 x 2.1 mm, 1.8 μ m (Agilent)	Triart C18, 50 x 0.3 mm, 1.9 μ m, (YMC)
Column	Zorbax RRHD SB-C18, 100 x 2.1 mm, 1.8 μ m (Agilent)	Triart C18, 50 x 0.3 mm, 1.9 μ m, (YMC)
Solvent flow rate	400 μ L/min	6 μ L/min
Injection volume	5 μ L	1 μ L
Nebulizing gas flow	10 L/min	0.4 L/min
Heat gas flow	12 L/min	3 L/min
LOD	≈ 7 pg	≈ 0.1 pg
LOQ	≈ 14 pg	≈ 0.7 pg

Shimadzu

→ Highly sensitive system → Unstability of retention time at low pressure
→ Able to work with low flow rate → Lake of robustness of the technique

Conclusion & Perspectives

- ✓ Development of a miniaturized sample preparation for targeted analysis of oxylipins
- ✓ Combinaison of MeOH/ACN/EA (60/30/10) was chosen as elution solvent for μ SPE
- ✓ Validation of the extraction method on other matrices (tissues, cells,...) to be done
- ✓ Robotisation of the sample preparation on robot TECAN

- ✓ Future analyses will be tested on μ HPLC-ESI-MS/MS, Shimadzu



FLUENT 780 TECAN

	Acquity UPLC CSH C18, 100 x 1.0 mm, 1.7 μ m (Waters)
Column	Acquity UPLC CSH C18, 100 x 1.0 mm, 1.7 μ m (Waters)
Solvent flow rate	100 μ L/min
Injection volume	2 μ L
Nebulizing gas flow	2 L/min
Heat gas flow	10 L/min
LOD	≈ 2 pg
LOQ	≈ 4 pg

References

- Oasis HLB 96-well μ Elution Plate, 2 mg Sorbent per Well, 30 μ m, Waters
- Oasis HLB 96-well Plate, 30 mg Sorbent per Well, 30 μ m, Waters

Abbreviations

SPE : Solid Phase Extraction
MeOH : Methanol, ACN : Acetonitrile, IPA : Iso-Propan-2-ol,
EA : Ethyl Acetate
ESI : Electro-Spray Ionisation
DMRM : Dynamic-Multiple Reaction Monitoring