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Development of a free oxylipins absolute quantification method on a microflow HPLC-microflow ESI-MS/MS system (μ HPLC- μ ESI-MS/MS)

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Anaëlle Durbec, Adrien Harmois, Nicolas Cenac, Pauline Le Faouder, Sascha Rexroth, et al.. Development of a free oxylipins absolute quantification method on a microflow HPLC-microflow ESI-MS/MS system (μ HPLC- μ ESI-MS/MS). Analytics2022, Sep 2022, Nantes (Cité des Congrès), France. . hal-04044958

HAL Id: hal-04044958

<https://hal.science/hal-04044958>

Submitted on 24 Mar 2023

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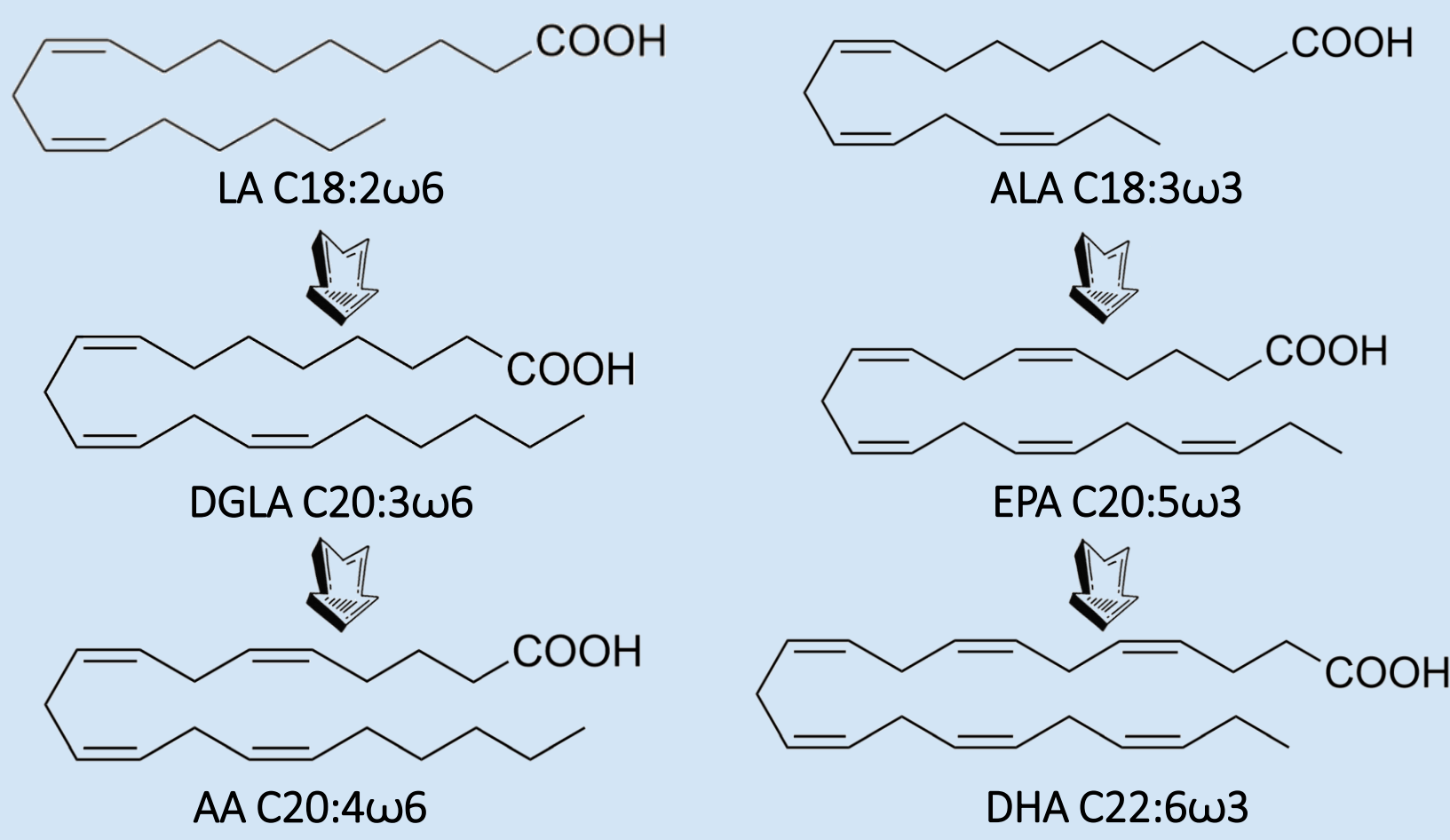
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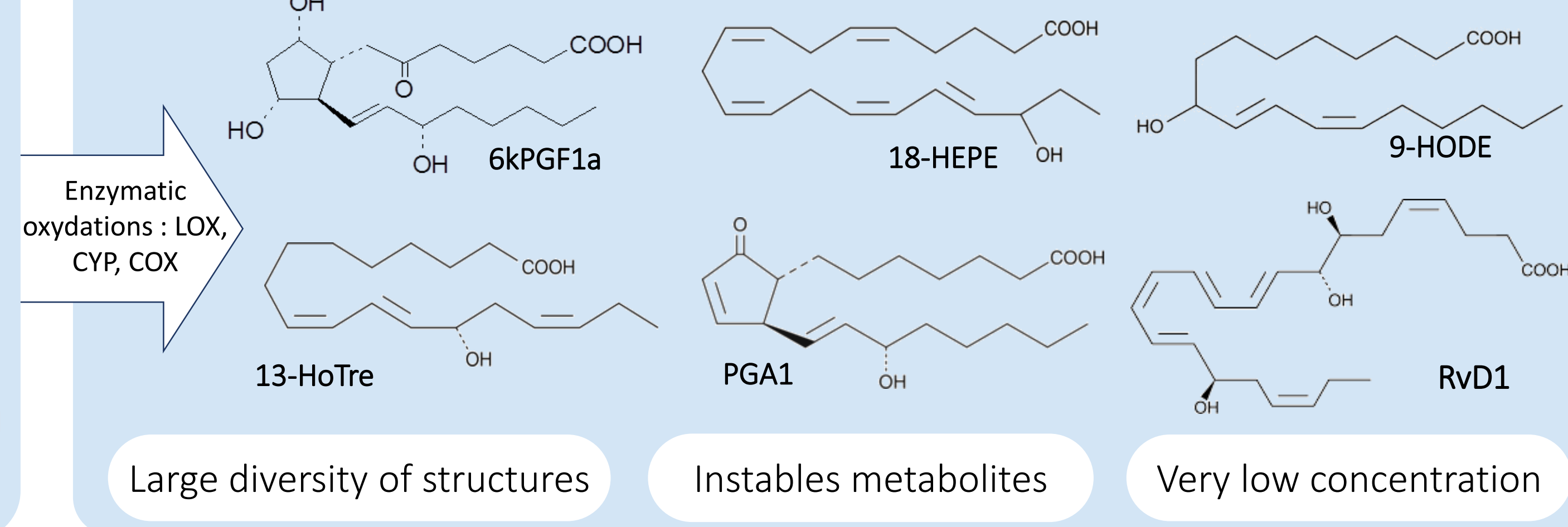
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From polyunsaturated fatty acids



Generation of free oxylipins



Biological role



Promotion, inhibition and regulation of inflammation and its resolution

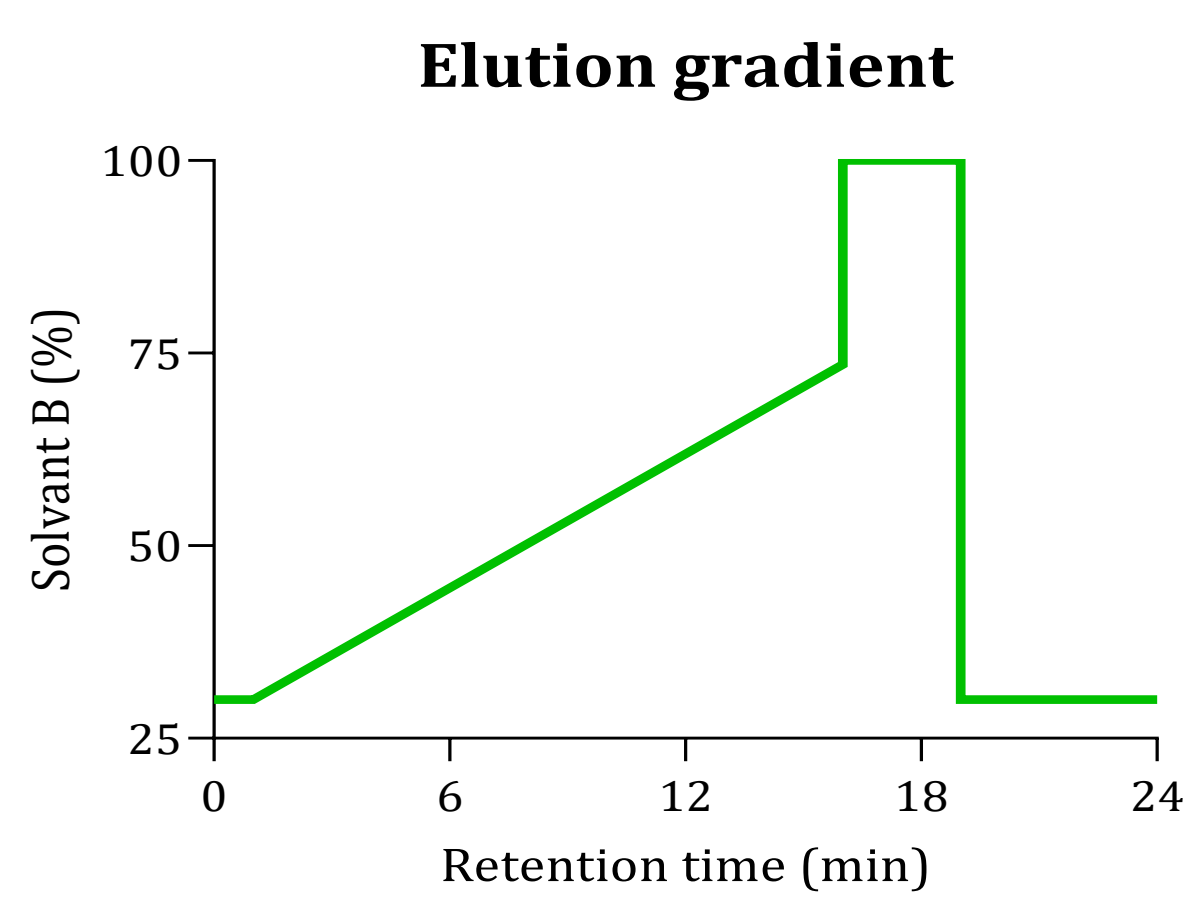
Aim of the work

Improve the current oxylipins profiling by miniaturizing the analysis

μ HPLC- μ ESI-MS/MS

μ HPLC parameters

Column: Triart-C18, 50 x 0.3 mm, YMC
Solvent A: H₂O, 0.1% formic acid
Solvent B: ACN, 0.1% formic acid
Flow rate: 6 μ L/min
Oven temperature: 40°C
Injection volume: 1 μ L



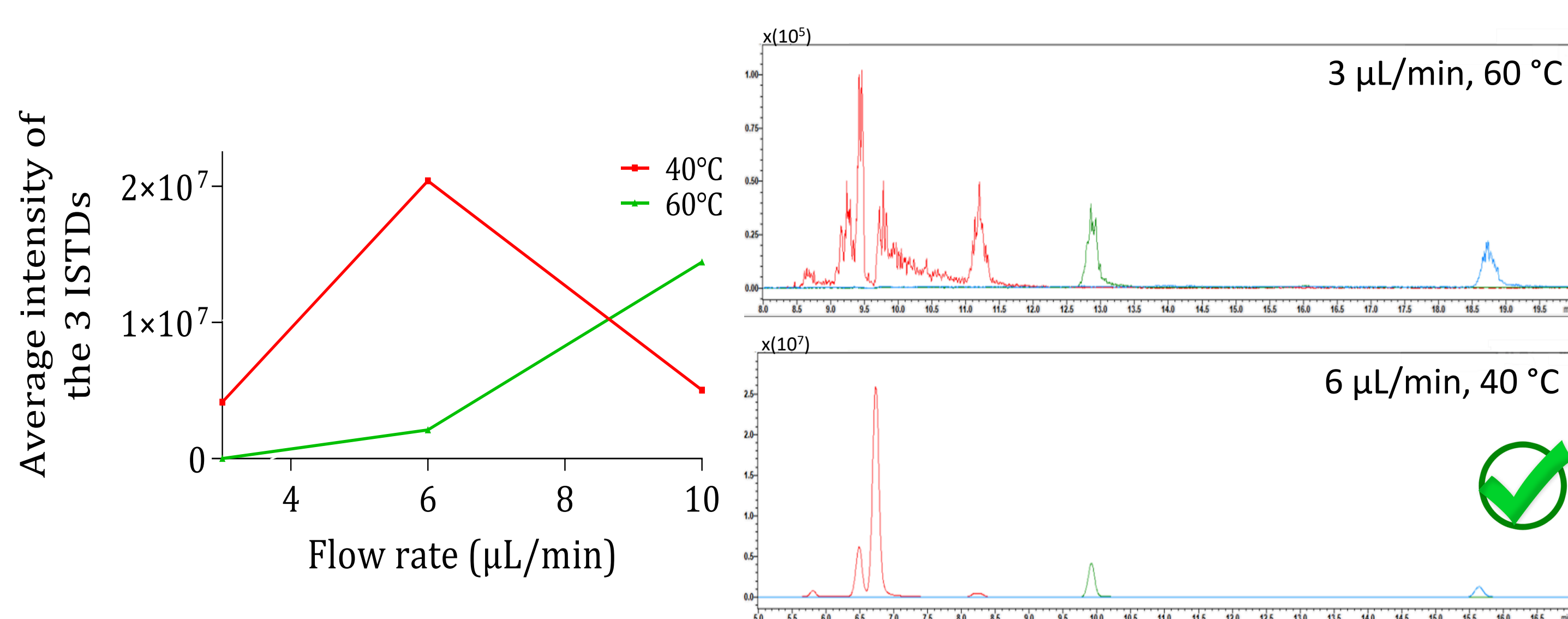
μ ESI-MS/MS parameters

Source optimisation on 3 ISTD
Negative mode [M-H]
Analyses in dynamic multiple reaction monitoring
56 transitions



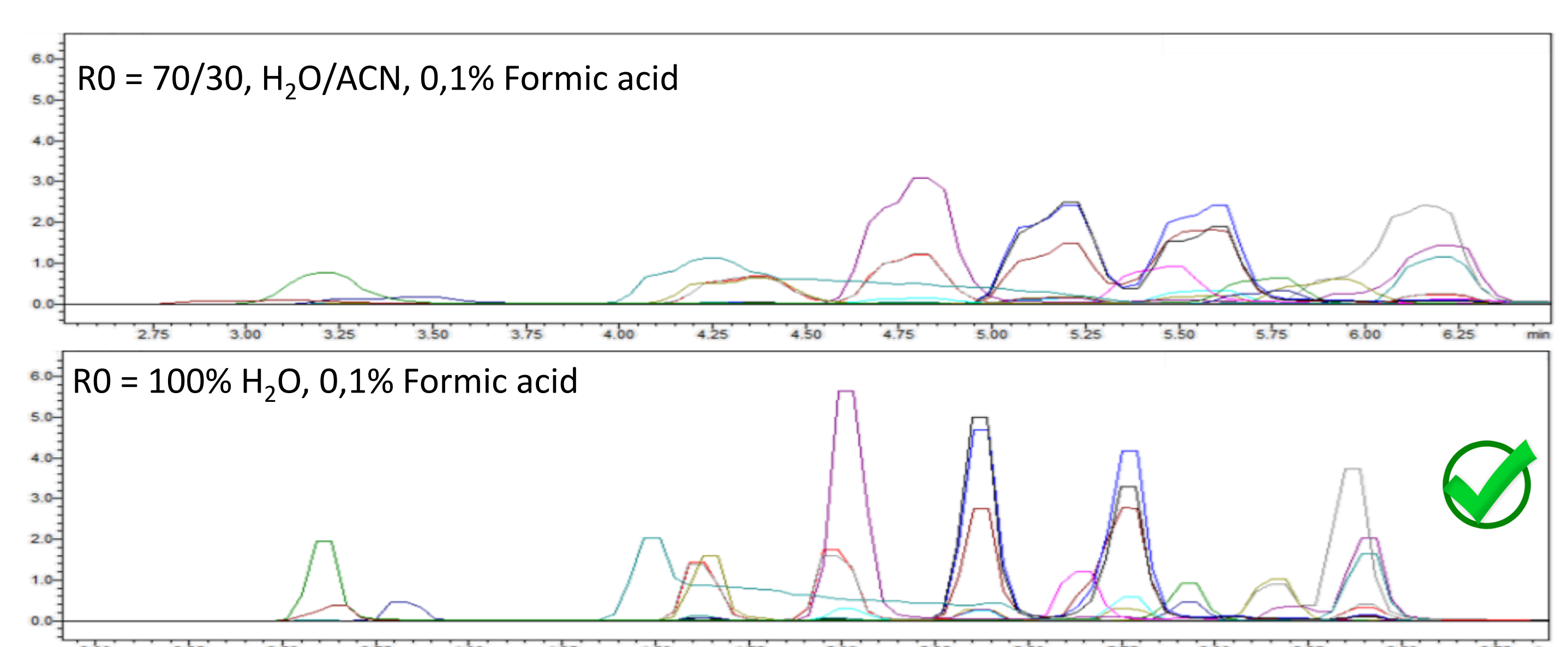
Nexera X2 Mikros - LCMS 8060, Shimadzu

Solvent flow rate and oven temperature optimisation on ISTDs



→ Loss of signal at 3 μ L/min and 60 °C
→ Gain of intensity at 6 μ L/min and 40 °C

Flushing solvent (R0) optimisation for the needle and the injection loop



→ Increasing the percentage of water in R0 reduce the hydrophobicity of the sample to ensure binding on the column

Comparison of three different systems



1290 Infinity - G6460 QqQ, Agilent

HPLC - ESI - MS/MS¹

| | |
|---------------------|---|
| Column | Zorbax RRHD SB-C18, 100 x 2.1 mm, 1.8 μ m (Agilent) |
| Solvent flow rate | 400 μ L/min |
| Injection volume | 5 μ L |
| Nebulizing gas flow | 10 L/min |
| Heat gas flow | 12 L/min |
| LOD | ≈ 7 pg |
| LOQ | ≈ 14 pg |



Nexera X2 Mikros - LCMS 8060, Shimadzu

μ HPLC - ESI - MS/MS

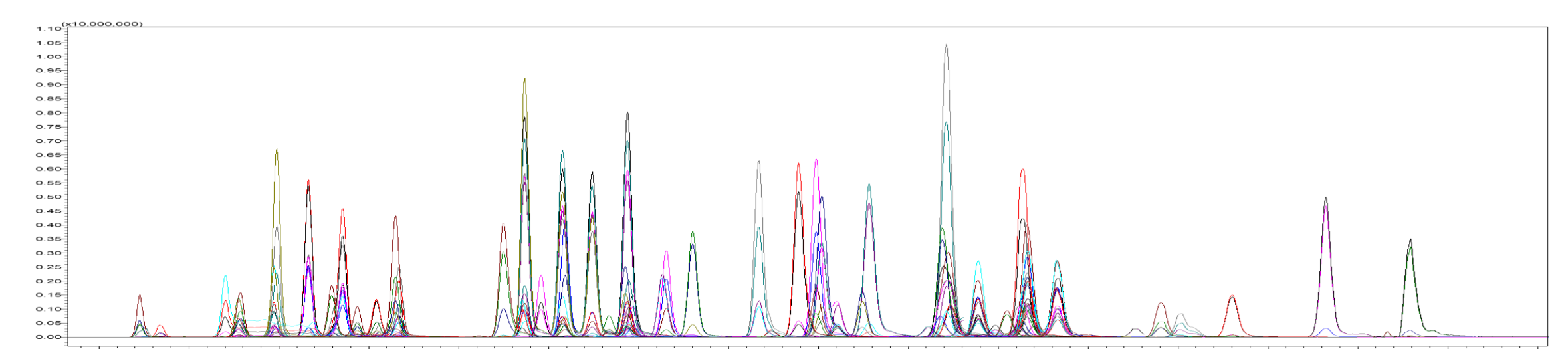
| | |
|---------------------|--|
| Column | Acquity UPLC CSH C18, 100 x 1.0 mm, 1.7 μ m (Waters) |
| Solvent flow rate | 100 μ L/min |
| Injection volume | 2 μ L |
| Nebulizing gas flow | 2 L/min |
| Heat gas flow | 10 L/min |
| LOD | ≈ 2 pg |
| LOQ | ≈ 4 pg |

μ HPLC - μ ESI - MS/MS

| | |
|---------------------|---|
| Column | Triart C18, 50 x 0.3 mm, 1.9 μ m, (YMC) |
| Solvent flow rate | 6 μ L/min |
| Injection volume | 1 μ L |
| Nebulizing gas flow | 0.4 L/min |
| Heat gas flow | 3 L/min |
| LOD | ≈ 0.1 pg |
| LOQ | ≈ 0.7 pg |

Conclusion

- ✓ Optimisation of a high sensitive targeted analysis method on μ HPLC- μ ESI-QqQ
- ✓ Using solvent A in R0 allows a better resolution of the most polar compounds
- ✓ The miniaturization of the system makes it possible to reduce the amount of sample extracted
- ✓ Quantification of 56 oxylipins in 24 minutes between 0.7 pg and 500 pg



References

¹ Le Faouder P et al, J Chrom B, 2013, vol 932 p123-133

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