Experimental design for the optimization of multi-residual analysis of oxygenated metabolites of PAHs (hydroxylated, quinones) in sediments

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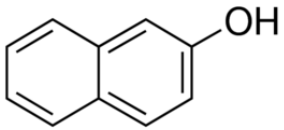
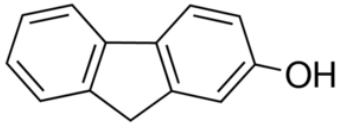
Contaminants

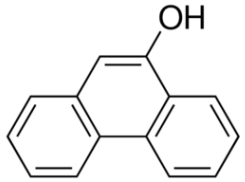
Hydroxy-PAHs (OH-PAHs):



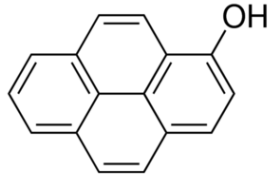
2- Naphthol



2- Hydroxyfluorene

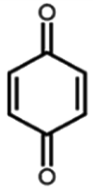


9- Phenanthrol

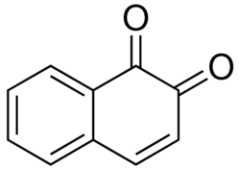


1- Hydroxypyrene

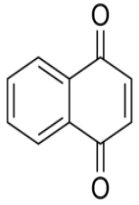
Quinones:



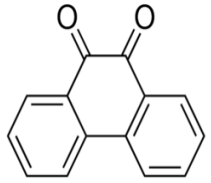
1,4- Benzoquinone



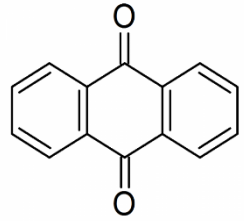
1,2- Naphthoquinone



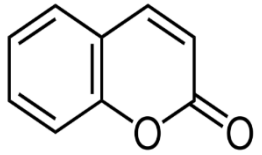
1,4- Naphthoquinone



9,10- Phenanthrenequinone



9,10- Anthraquinone



Coumarin

- No standardized methods for oxygenated PAHs (oxy PAHs)

Matrix

Sediment modelling a natural sediment from a Normand harbors

Silt: ~70%

Clay: ~20%

Sand: <5%

Organic matter: 2,5-10%

- **Difficult**
  - **Organic matter**
  - **Finest particles**

OBJECTIVES

- **Develop a method to extract simultaneously a mixture of four hydroxylated PAHs (OH-PAHs) and six carbonyl PAHs (quinones) from sediments (MAE)**

- **Develop a method to analyze these compounds at trace levels (GC-MS and HPLC-FLD/UV)**

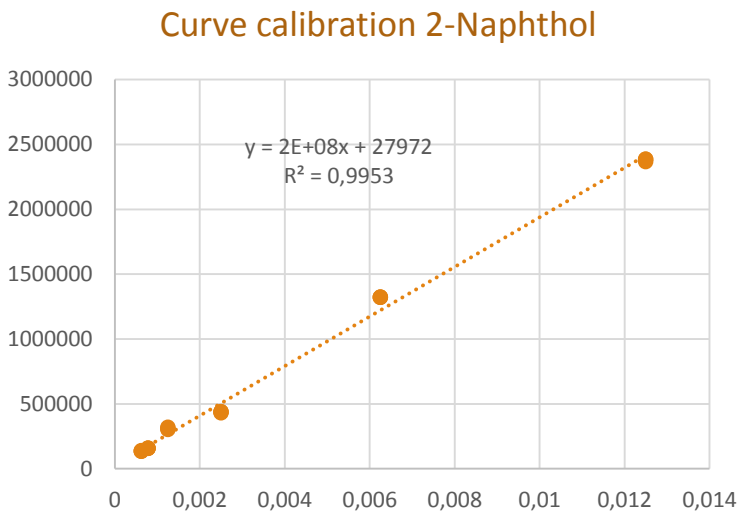
2) Simultaneous MAE extraction and analyzes of two families of oxygenated PAHs

a) Choice of chromatographic analytical tools

Choice of analytical tools

HPLC – UV/FLD (coupled) → Low limit of detection (LOD) and limit of quantification (LOQ)

Quinones Hydroxy-PAHs



Quinones
LOD:2,4 - 4,3 µg/L
LOQ: 8,0- 14,2 µg/L

Hydroxy-PAHs
LOD: 0,2 - 0,3 µg/L
LOQ: 0,6- 1,0 µg/L

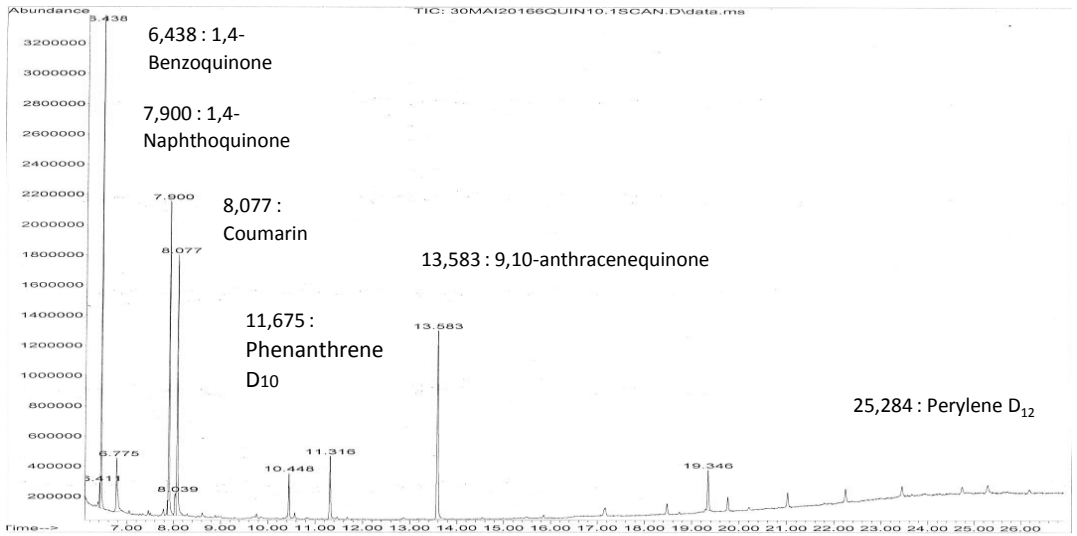
$LOD = 3.3 \times S_y / k$

$LOQ = 10 \times S_y / k$

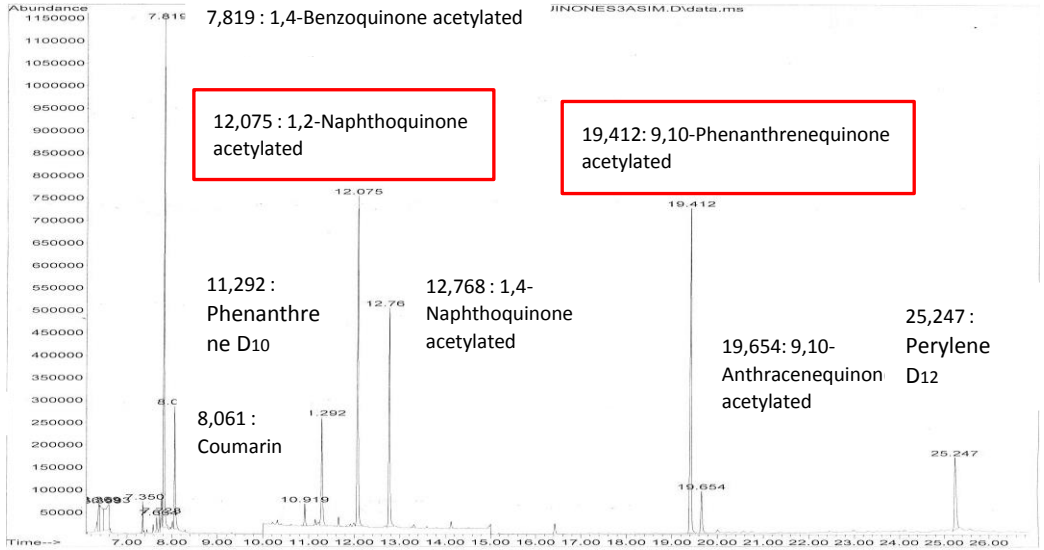
Legend:
K: slope of the calibration curve
S_y: standard error of the predicted y-value for each x-value

GC-MS

Without derivatization (quinones)



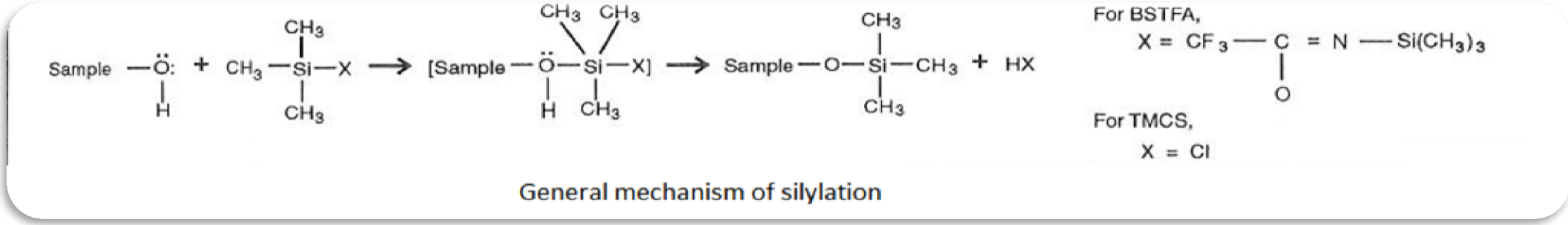
With derivatization (quinones)



GC-MS

- Silylation
- Acetylation

Silylation of hydroxy-PAHs



General mechanism of silylation

Catalysts: Pyridine and ethyl acetate

Best conditions: BSTFA+ TMCS, pyridine and ethyl acetate in 5 minutes of reaction

Time(min): 5, 15, 30, 45 and 60

LOD: 90,0-220,0 µg/L **LOQ:** 300,0-720,0 µg/L ⟶ With derivatization

LOD: 180,0- 600,0µg/L **LOQ:** 610,0-2000,0 µg/L ⟶ Without derivatization

➞ **Sensitivity improved by a factor 3**

Acetylation of quinones

- **1000µL** solution of quinones
- **0,1g** Zn
- **400µL** acetic anhydride

Cooling to room temperature

Heating 80°C, **15 min**

Centrifugation 2000 rpm, 10 min

Take the above organic phase

Add 60µL octanol

Organic phase evaporated (under N2 flow)

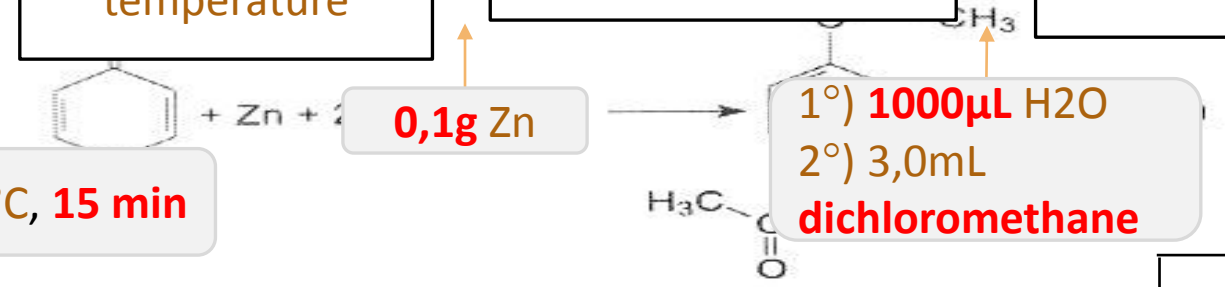
Redissolved in acetonitrile until **1000µL**+10µL internal standard

Aqueous phase discarted

Heating 80°C, **15 min**

0,1g Zn

1°) **1000µL** H2O
2°) 3,0mL **dichloromethane**



Acetylation reaction of 1,4-benzoquin

LOD: 190,0-290,0µg/L **LOQ:** 640,0-960,0µg/L → With derivatization

LOD: 560,0-10000,0µg/L **LOQ:** 850,0-33000,0µg/L → Without derivatization

➡ **Sensitivity improved by a factor 4-53** → Ortho quinones

2) Simultaneous MAE extraction and analyzes of two families of oxygenated PAHs

b) Optimization of MAE extraction by experimental design

Microwave assisted extraction

	MAE	Soxhlet	Sonication
Time of extraction	3- 30 min	3-48hrs	10-60min
Sample amount	1-10g	1-30g	1-30g
Solvent volume	10-40mL	100-500mL	30-200mL

ADVANTAGES!!

- **MAE never tested for quinones and hydroxy-PAHs**

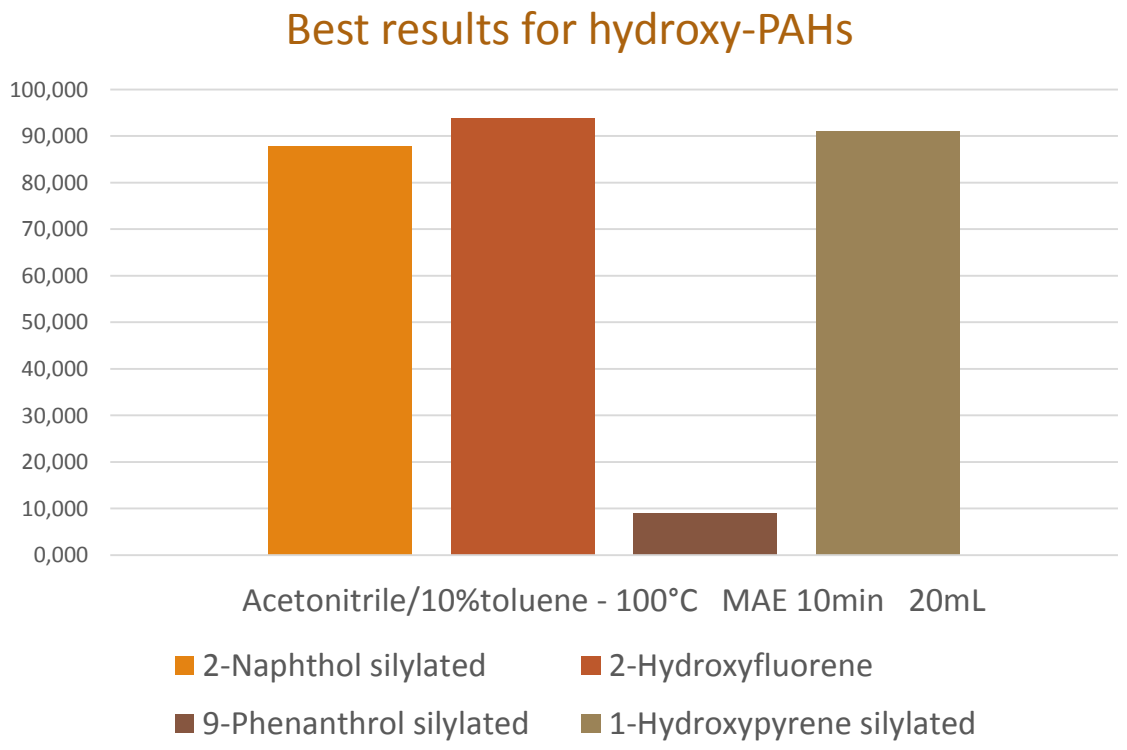
Microwave assisted extraction

➡ **First trials**

- **Volume(mL):** 10 and 20
- **Temperature(°C):** 80, 100 and 120
- **Solvent:**

- Acetonitrile
- 90%Acetonitrile/10%toluene*
- 90% Acetonitrile/10%dichloromethane
- 50% Acetone/50%toluene**

Time(min): 10, 20 and 30



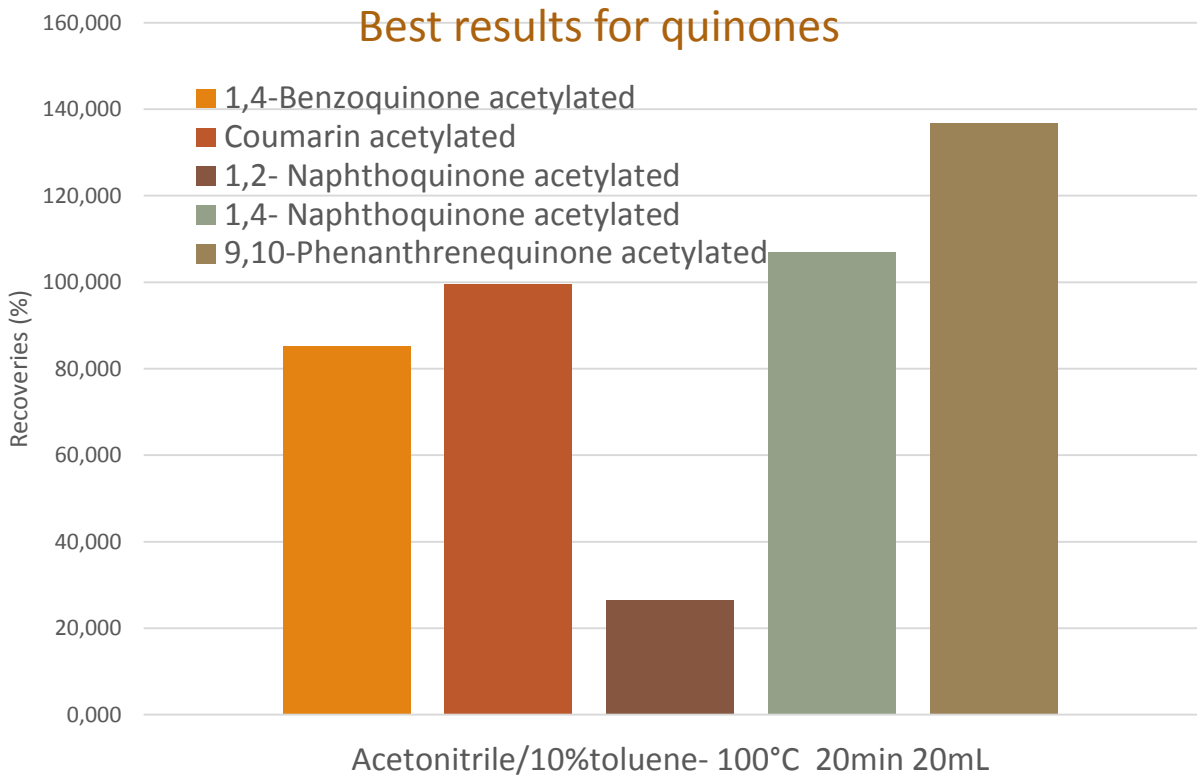
*Oriol et al., Anal. Methods, 2013, 5, 6297-6305
**Optimal for PAHs

Microwave assisted extraction

- **Volume(mL):** 10 and 20
- **Temperature(°C):** 80, 100 and 120
- **Solvent:**

- Acetonitrile
- 90%Acetonitrile/10%toluene
- 90% Acetonitrile/10%dichloromethane
- 50% Acetone/50%toluene

Time(min): 10, 20 and 30



Microwave assisted extraction

- **Not the same conditions of extraction for the two families**

- **Univariate optimization not appropriate → chemometric approach to find the influent factors, their interactions and a compromise for the two families**

First experimental design: fractional factorial design 2^{4-1}

Tests	Temperature Extraction	Volume solvent	Nature solvent	Time extraction
1	80°C (-1)	10mL (-1)	CH3CN/10%CH2CL2 (-1)	10min (-10)
2	80°C (-1)	10mL (-1)	CH3CN/10%toluene (+1)	30min (+1)
3	80°C (-1)	30mL (+1)	CH3CN/10%CH2CL2 (-1)	30min (+1)
4	80°C (-1)	30mL (+1)	CH3CN/10%toluene (+1)	10min (-10)
5	120°C (+1)	10mL (-1)	CH3CN/10%CH2CL2 (-1)	30min (+1)
6	120°C (+1)	10mL (-1)	CH3CN/10%toluene (+1)	10min (-10)
7	120°C (+1)	30mL (+1)	CH3CN/10%CH2CL2 (-1)	10min (-10)
8	120°C (+1)	30mL (+1)	CH3CN/10%toluene (+1)	30min (+1)
9 - 15	100°C (0)	20mL (0)	CH3CN (0)	20min (0)

- **Screening design**

➡ **Influent factors and possible interactions?**

- **2 levels + 0 center points**

- **Results (recovery yields):**

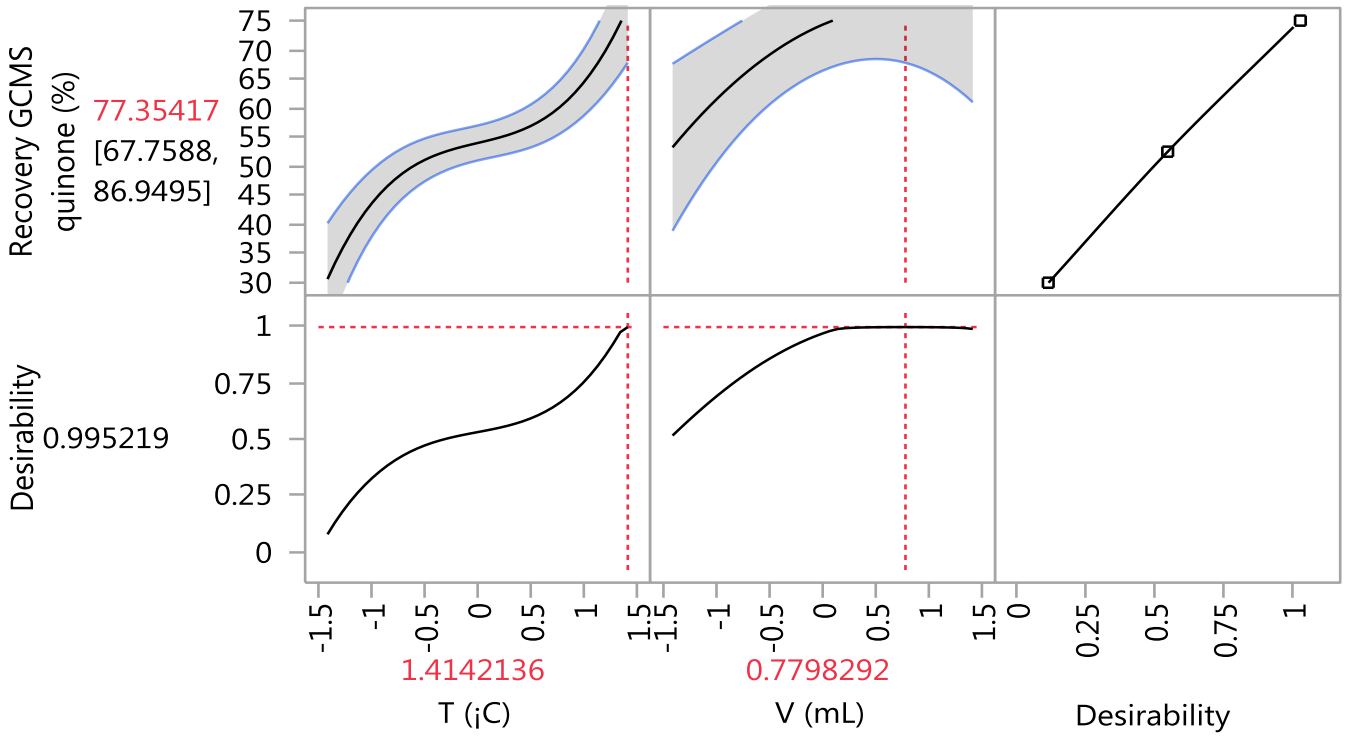
1. **Most influent factors:** Temperature and volume
2. **Not influent:** Time set to 10 minutes
3. **Solvent:** compromise for the two families ➡ Acetonitrile/Dichloromethane 90/10

Second Experimental design: central composite design 2^2

Tests	Temperature Extraction	Volume solvent
1	80°C (-1)	15mL (-1)
2	80°C (-1)	35mL (+1)
3	120°C (+1)	15mL (-1)
4	120°C (+1)	35mL (+1)
5	72°C (-α)	25mL (0)
6	128°C (+α)	25mL (0)
7	100°C (0)	11mL (-α)
8	100°C (0)	39mL (+α)
9- 13	100°C (0)	25mL (0)

- Surface response design only to temperature and volume studied
- 5 levels ➡ non linear modeling

Second Experimental design: central composite design 2^2

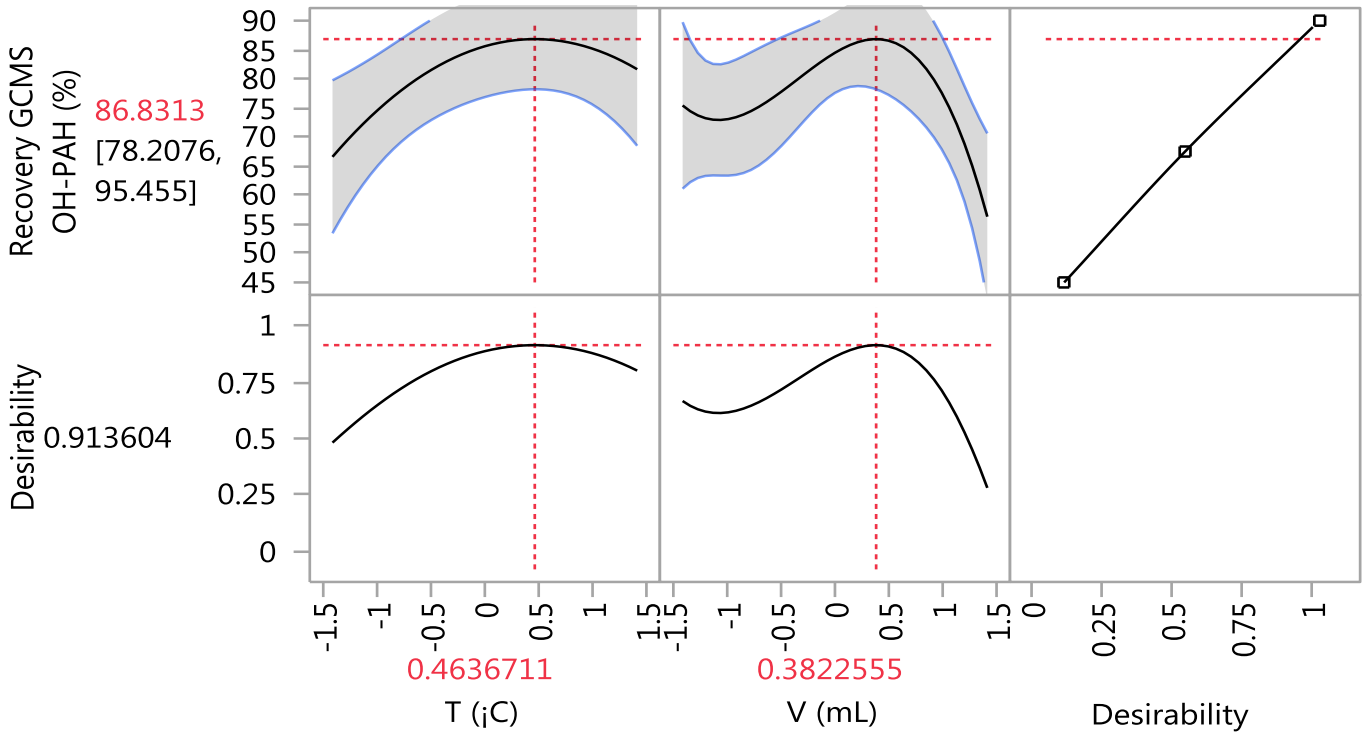


Response surface for quinones

- **Best conditions**

T = + 1,41 (128ºC)
V = + 0,78 (33mL)

Second Experimental design: central composite design 2^2

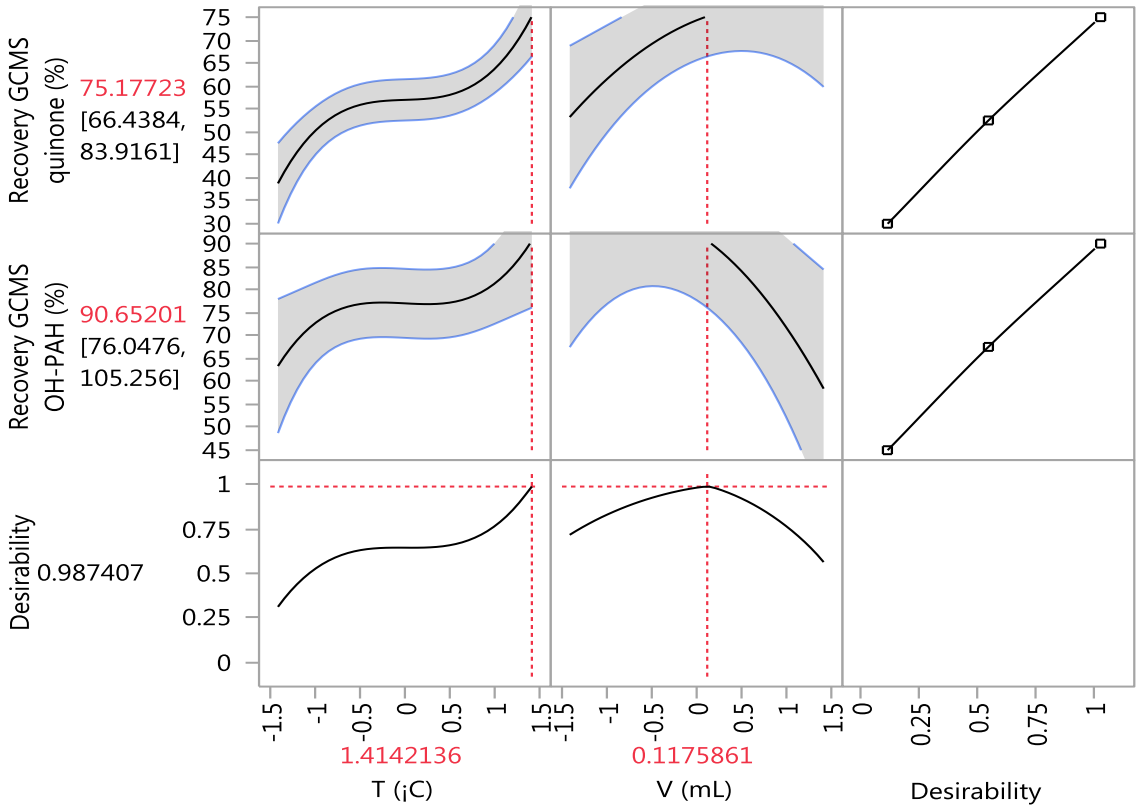


Response surface for hydroxy-PAHs

- **Best conditions**

  T = + 0,46 (110ºC)
  V = + 0,38 (29mL)

Second Experimental design: central composite design 2



Together fitted

- **Influent factors**

$T^3 > V^2 > T \times V$

- **Best conditions for the two families**

T= 128ºC (+ α)

V= 26mL (+0,12)

4) Conclusion and perspectives

CONCLUSION

- Derivatizations before GC-MS improve the detection of the hydroxy-PAHs and quinones (particularly ortho-quinones)

- HPLC-UV/FLD is more sensitive than GC-MS but GC-MS allows unknown compounds

- The best conditions for the extraction of two oxygenated families were found for MAE (time, solvent, temperature and volume) ⟶ need to validate the method MAE- GC-MS

PERSPECTIVES

- Modeling of MAE - HPLC-UV/FLD to do

- Comparison of the two methods MAE – GC-MS and MAE – HPLC – UV/FLD

Thank you!

Questions?

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