KU LEUVEN

Online LC x LC coupled to high-resolution mass spectrometry for the characterization of pharmaceutical residues in hospital wastewater

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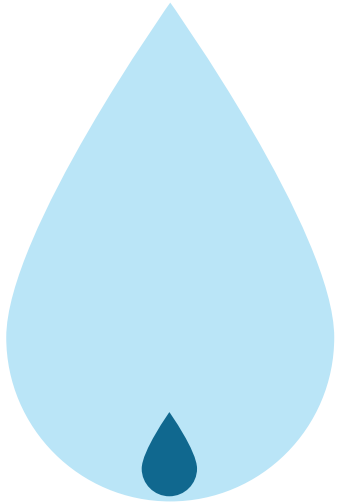
Global freshwater availability

71% of Earth's surface covered in water

2.5% Freshwater (mostly locked up in ice and underground)

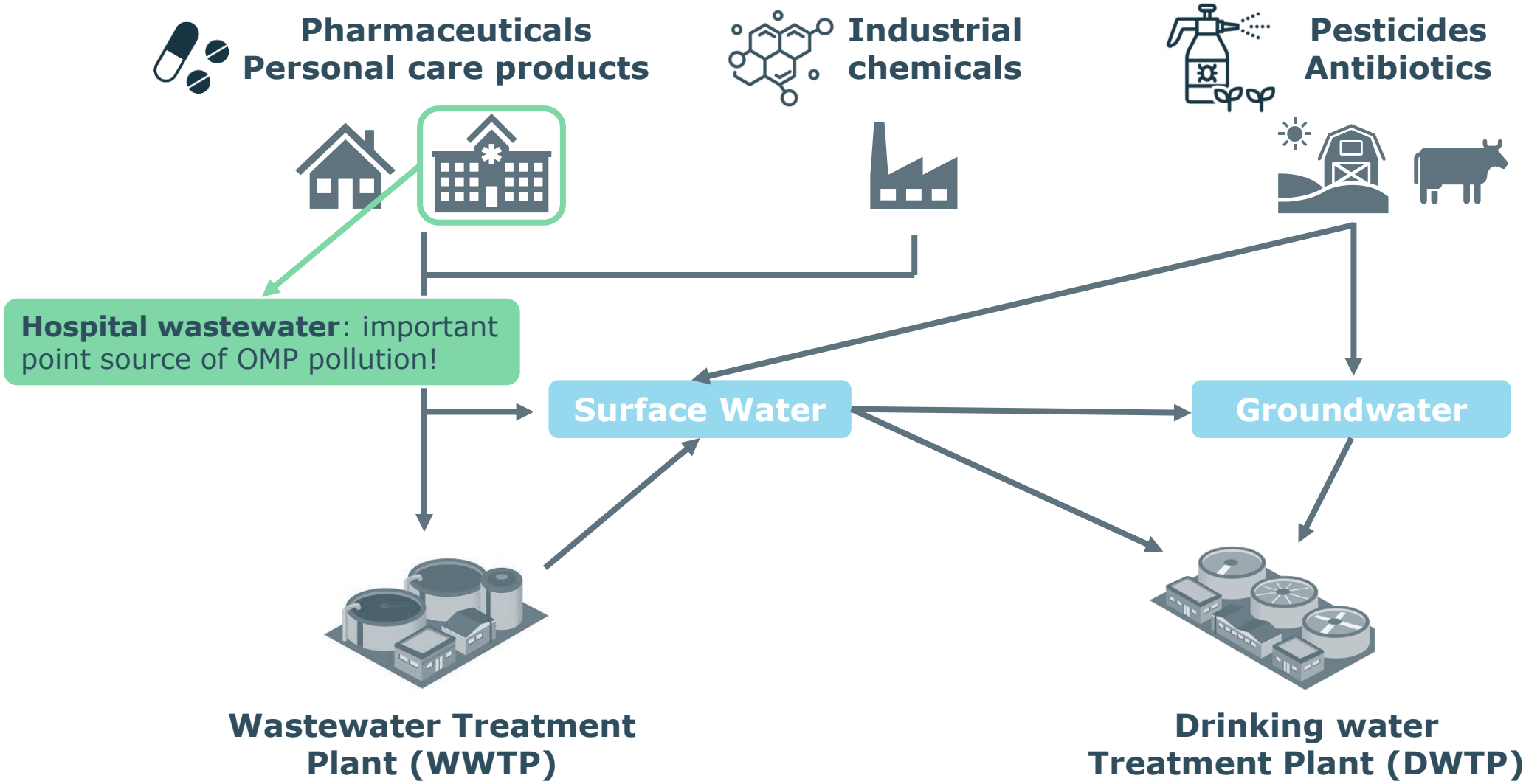


1% Readily available freshwater

Availability under ↑ pressure:

- Climate change
- Increasing demands
- **Pollution**

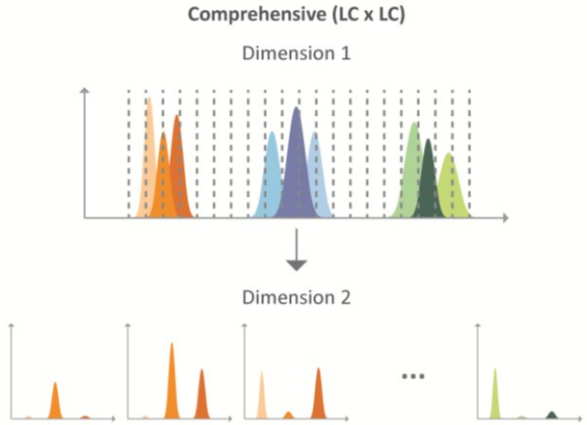
Organic micropollutants (OMPs)



How to identify pharmaceuticals in hospital wastewater?

Problem:
- Highly complex samples
- Low analyte concentrations

Possible solution: online LC x LC coupled to HR-MS

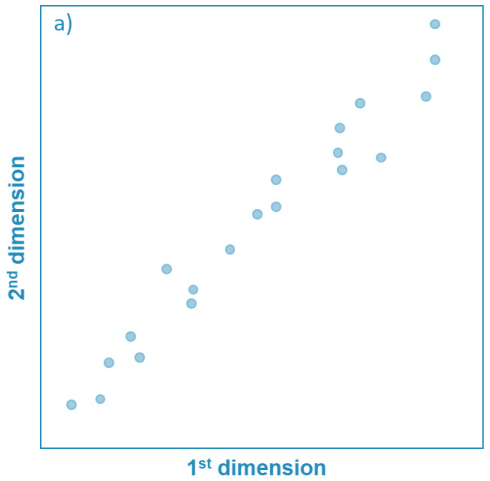


*Submitted recently in JCA

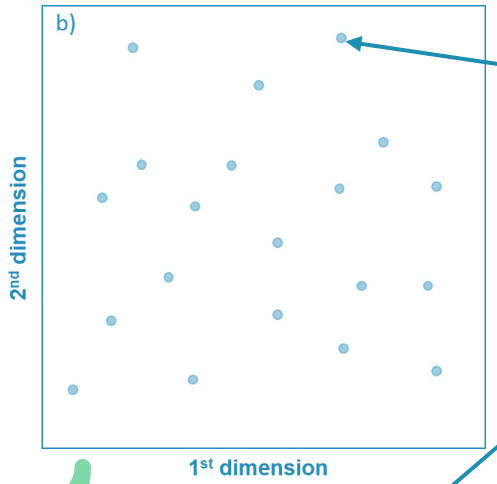
Online LC x LC: complex method development!

Which combination of ^{1}D and ^{2}D conditions?

Python-based tool (developed by S. Chapel)*:
Automated calculation of an **orthogonality score**
(based on multiple orthogonality metrics commonly applied in 2D-LC)



Low score



High score

Input:
Normalized retention times of target compounds under various LC modes/conditions

Output:
Orthogonality score of each possible combination

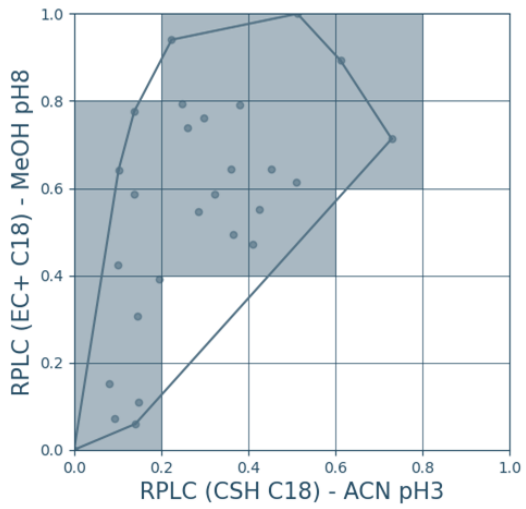
→ Final selection of conditions based on good orthogonality score, peak capacity and feasibility

Online LC x LC for hospital samples

Scouting experiments → retention data of 27 pharmaceuticals for 38 conditions
→ 703 possible combinations
→ 3 interesting options selected

	MODE	ORTHOGONALITY SCORE	THEORETICAL PEAK CAPACITY	PRACTICAL PEAK CAPACITY	^{1}D	^{2}D
1	RPLC x RPLC	0.42	14785	6210	EC C18 - MeOH pH8	CSH C18 - ACN pH3
2	RPLC x HILIC	0.57	5420	3089	EC C18 - MeOH pH8	BEH HILIC – ACN pH3
3	HILIC x RPLC	0.41	4902	2010	BEH HILIC – ACN pH3	CSH C18 - ACN pH3

1



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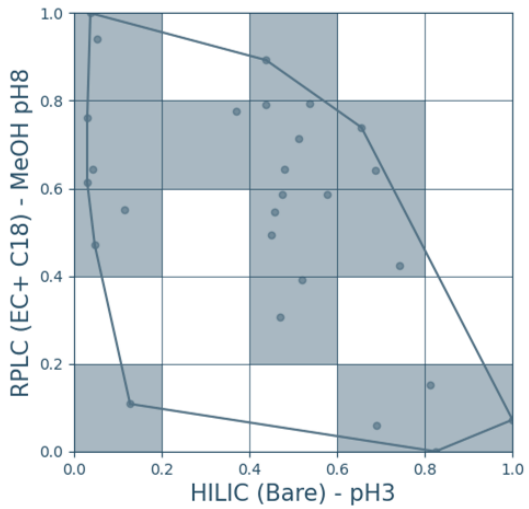

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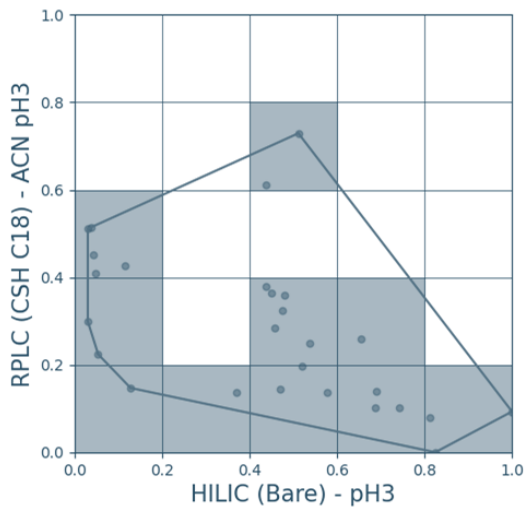
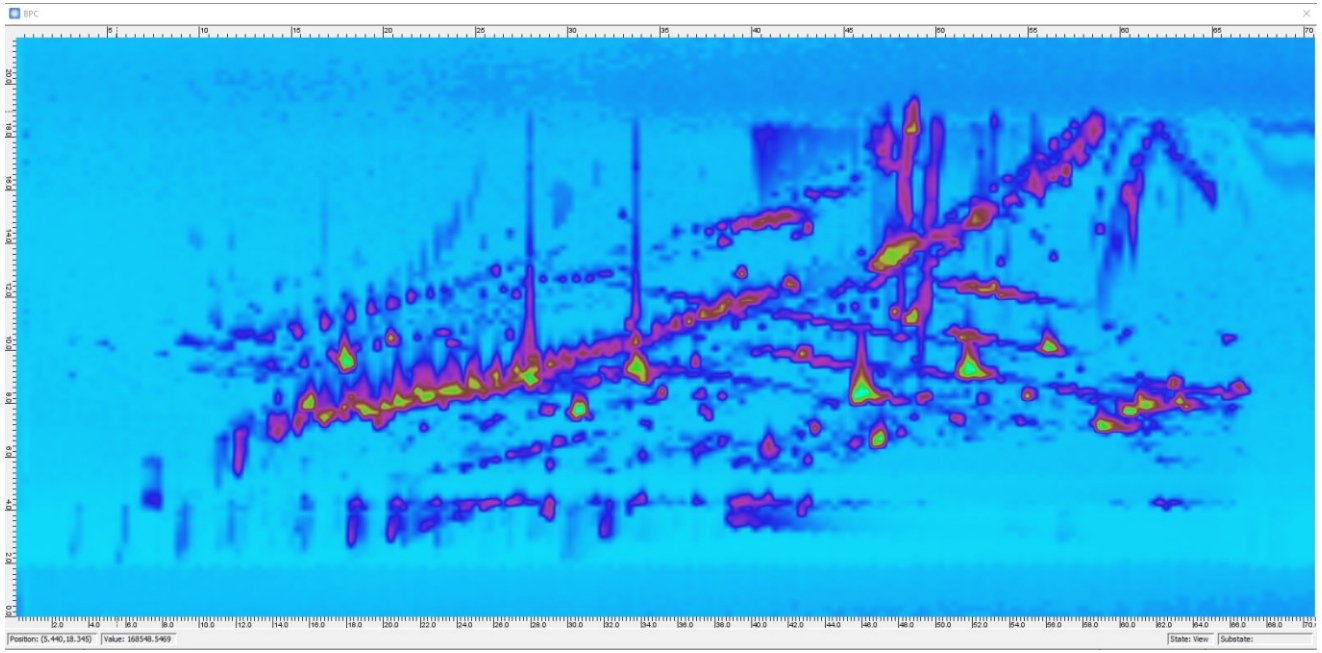


Most promising: RPLC x RPLC

	MODE	ORTHOGONALITY SCORE	THEORETICAL PEAK CAPACITY	PRACTICAL PEAK CAPACITY	^{1}D	^{2}D
1	RPLC x RPLC	0.42	14785	6210	EC C18 - MeOH pH8	CSH C18 - ACN pH3

UZ Leuven wastewater:



^{2}D (CSH C18 – ACN pH 3)

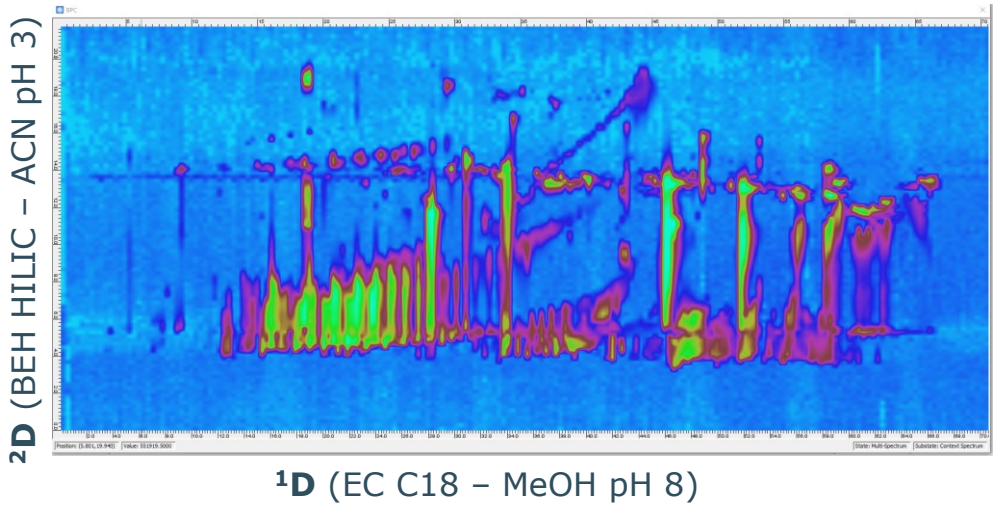
^{1}D (EC C18 – MeOH pH 8)

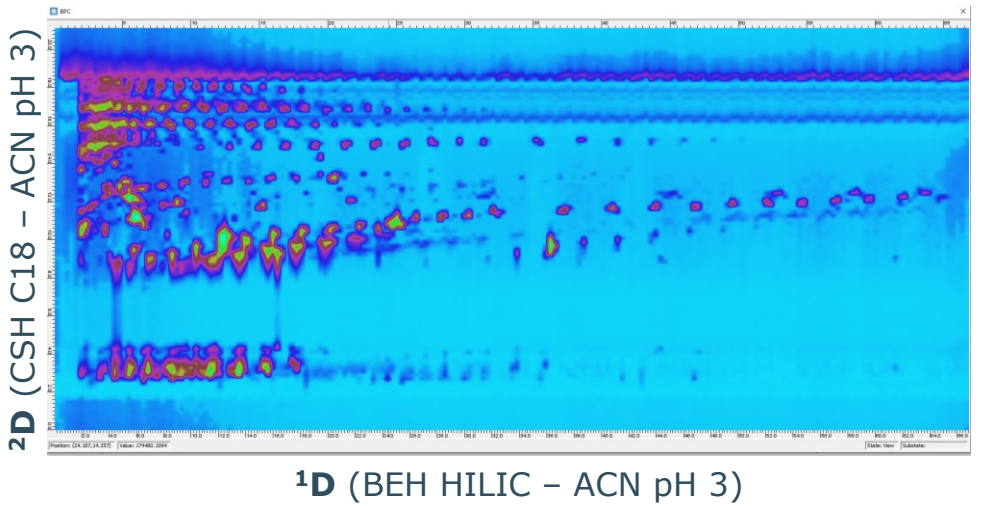
- Increased surface coverage by shifted gradient program
- Good ^{2}D peak shapes
- Succesful identification of 35 pharmaceuticals (HR-MS)
- Untargeted screening possible

→ Highly suitable method for analysis of complex hospital wastewater samples

Other options: HILIC x RPLC

	MODE	ORTHOGONALITY SCORE	THEORETICAL PEAK CAPACITY	PRACTICAL PEAK CAPACITY	^{1}D	^{2}D
2	RPLC x HILIC	0.57	5420	3089	EC C18 - MeOH pH8	BEH HILIC – ACN pH3
3	HILIC x RPLC	0.41	4902	2010	BEH HILIC – ACN pH3	CSH C18 - ACN pH3





Option 2: HILIC in ^{2}D
Peak shape issues, even with active solvent modulation (ASM)

Option 3: HILIC in ^{1}D
Not enough retention on HILIC column
→ Surface coverage not great

→ Additional method optimization required

Conclusions

- Hospital wastewater: **important source of water pollution** → suitable analytical methods required
- **Online LC x LC:** promising solution, but complex method development
- **Predicted orthogonality score:** useful in method development for selection of ^{1}D and ^{2}D conditions
- **RPLC x RPLC:** easier and efficient implementation
- **HILIC x RPLC:** more optimization required

→ **Online LC x LC coupled to HR-MS:** suitable method for analysis of pharmaceutical residues in hospital wastewater

Thank you for your attention!