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Metabolomics analysis of plasma with an extended coverage using two-dimensional liquid chromatography mass spectrometry

Carla ORLANDI

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INRAE ToxAlim

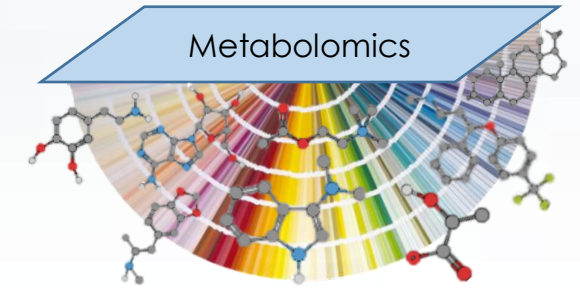
Toulouse



Context

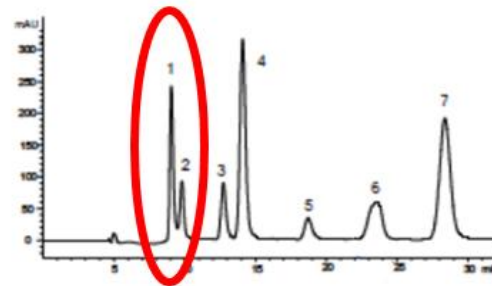
🔬 Increase metabolomic coverage in high throughput LC-HRMS analysis

🔬 Miniaturization to μ LC methods



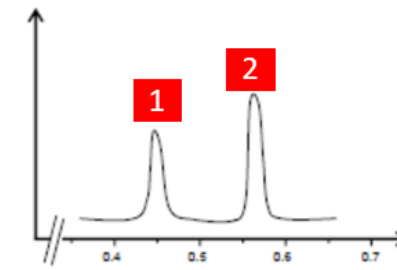
- Improve separation of co-eluting compounds ?
- Reduce the volume of injected sample and the number of injections ?
- Lower solvent consumption ?
- Enhance the speed of analytical methods ?
- Offer a global analysis ?

Heart-Cutting



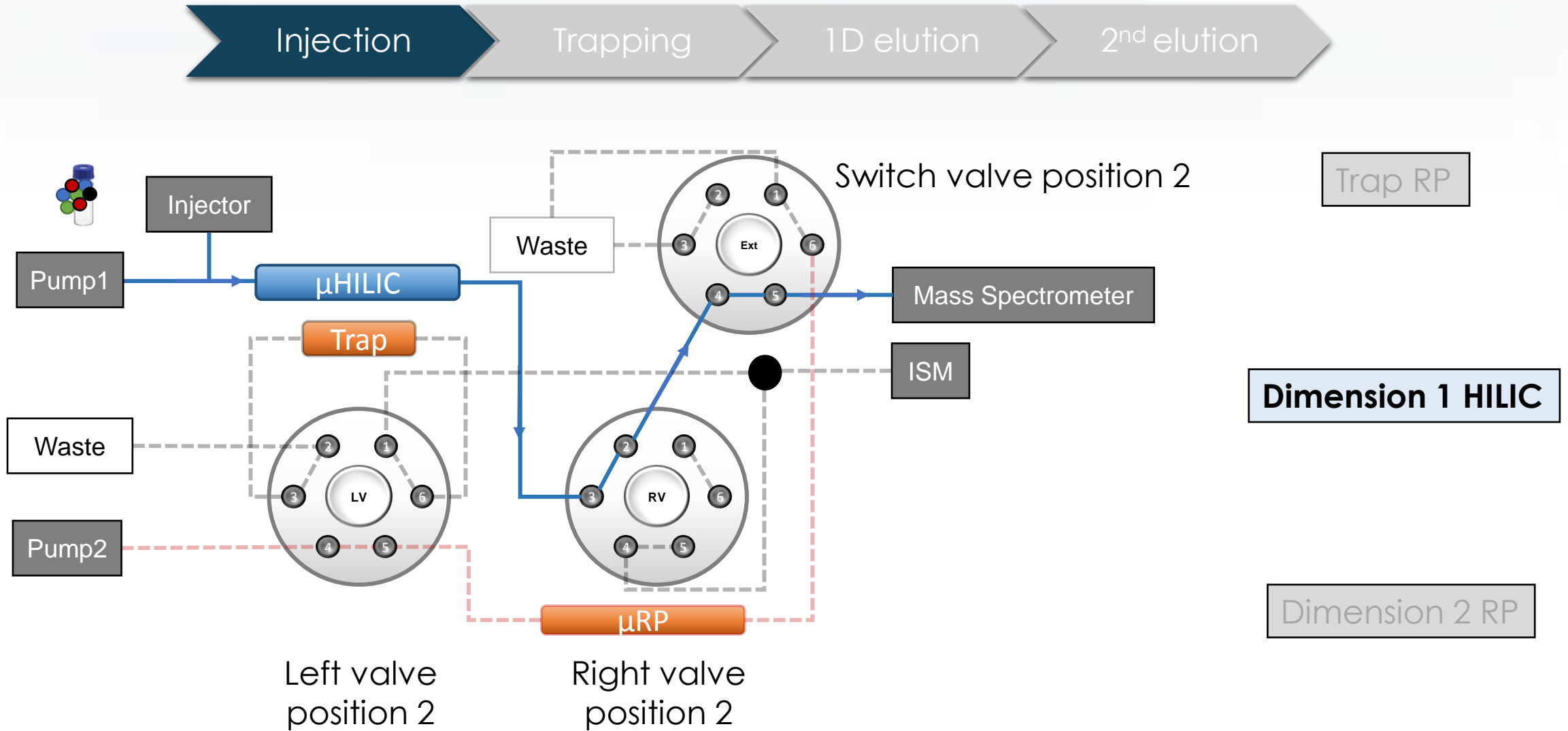
Dimension 1
 μ Hydrophilic Interaction Liquid Chromatography (HILIC)

Trap RP

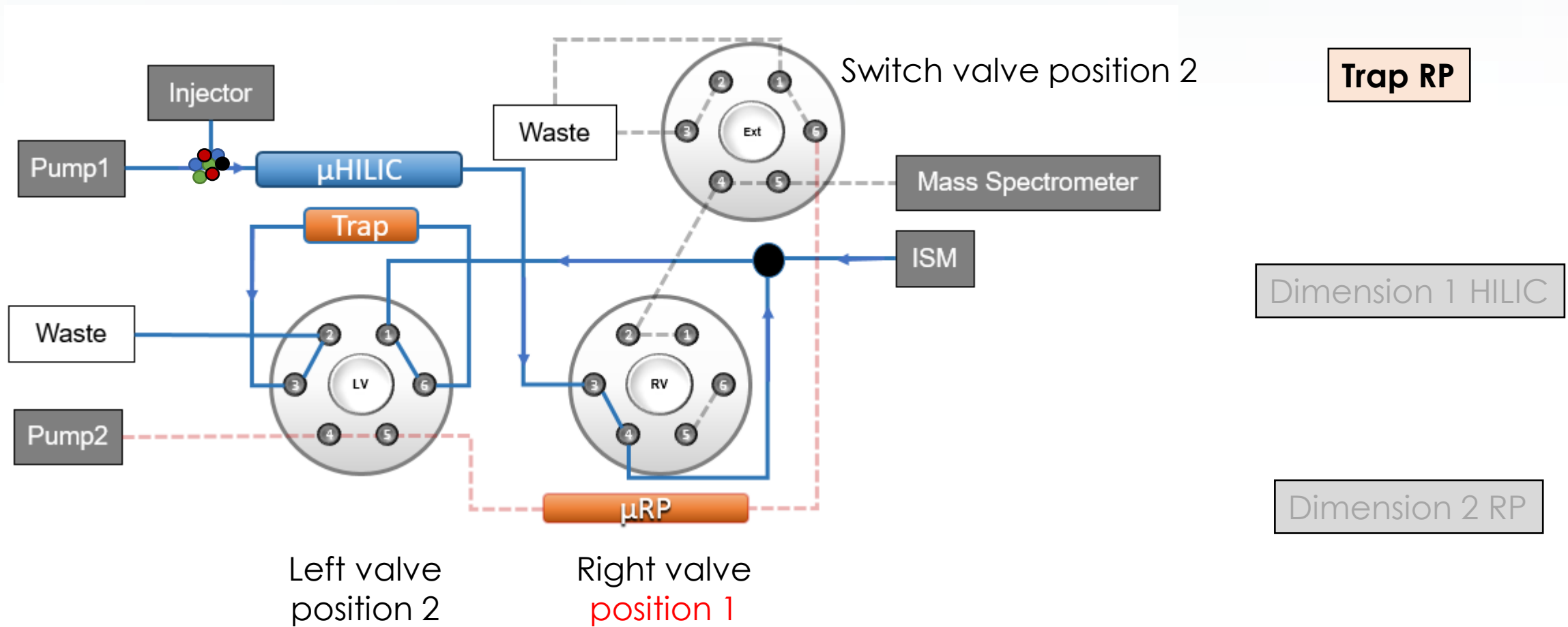


Dimension 2
 μ Reversed-Phase Liquid Chromatography (RP)

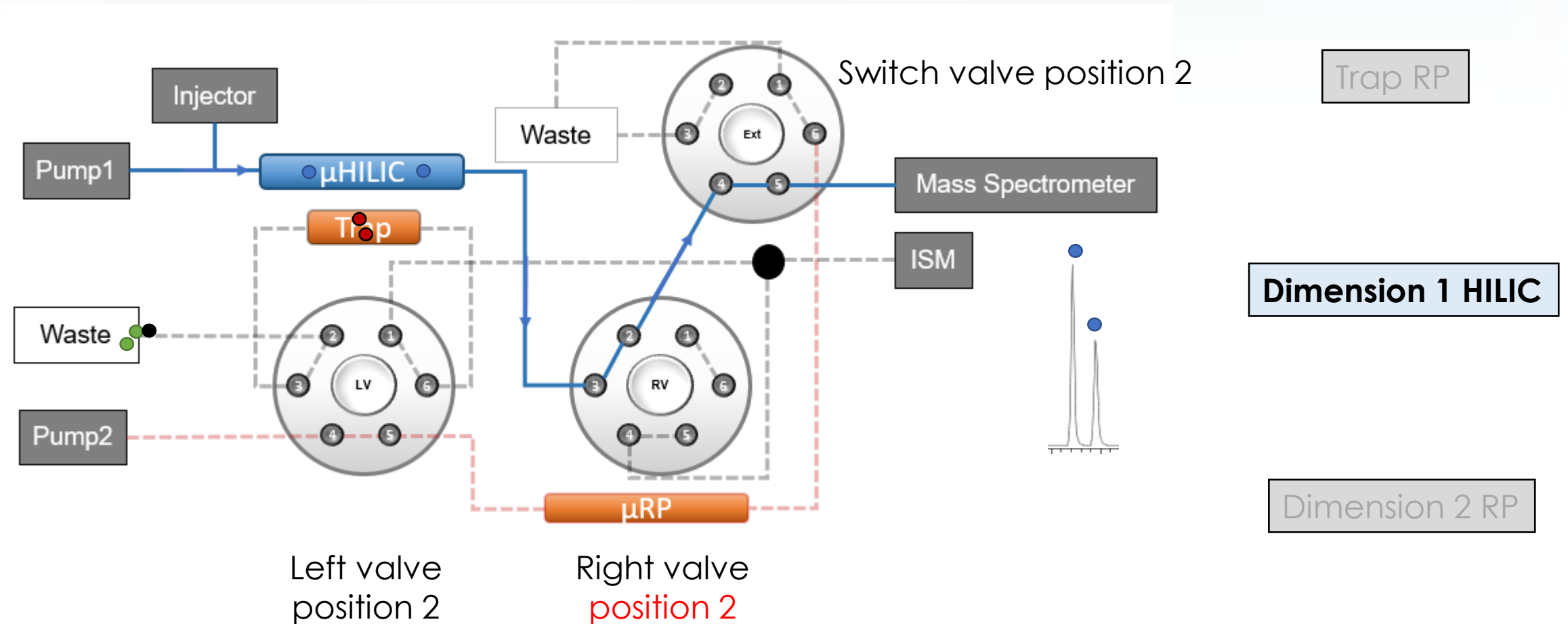
Heart-cutting approach: sample injection



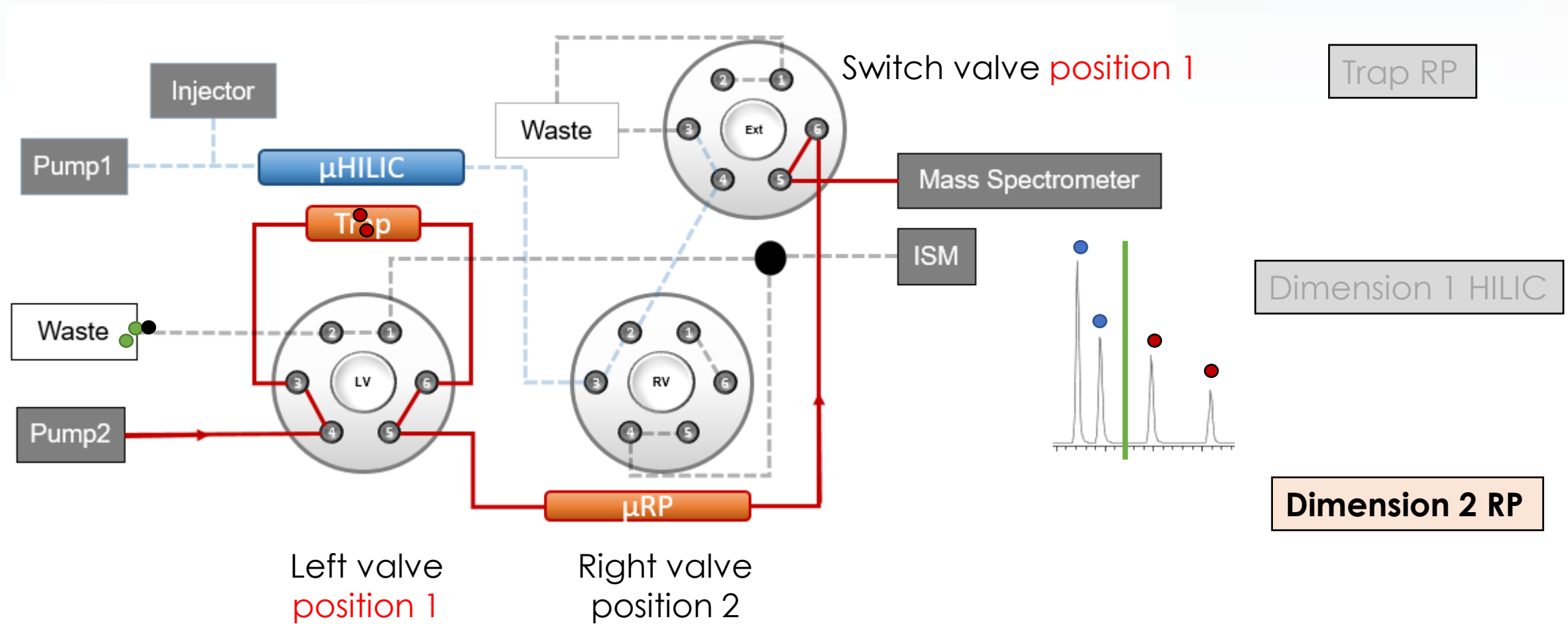
Heart-cutting approach: trapping step



Heart-cutting approach: 1st dimension μ HILIC analysis

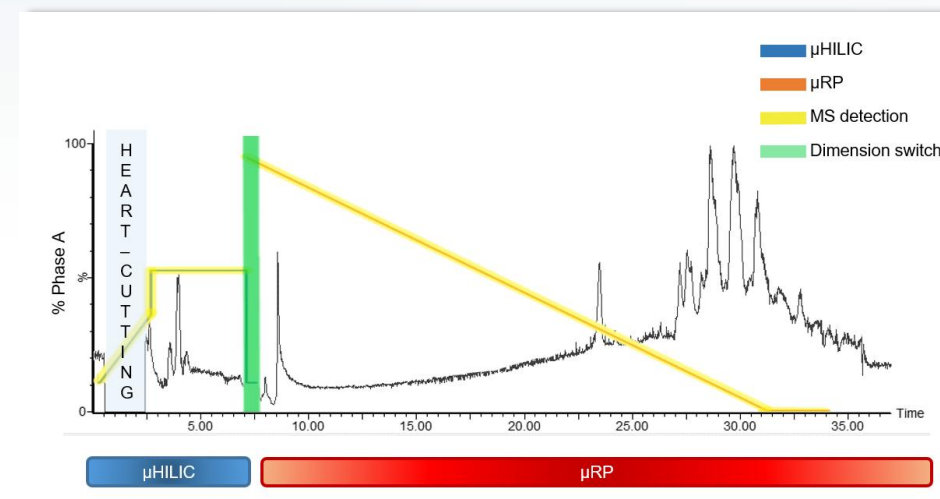


Heart-cutting approach: elution and 2 dimension μ RP analysis



Chromatographic and ionization conditions

	2D μ LC-MS	
	1D μ HILIC-MS	1D μ RP-MS
Mobile phase A	10mM ammonium formate pH 10,5 (NH ₄ OH)	H ₂ O/MeOH/HCOOH (95/5/0,1)
Mobile phase B	ACN 100%	MeOH/HCOOH (100/0,1)
Flow rate	0,1 mL/min	0,1 mL/min
Wash A2 B2	ACN/ H ₂ O (75/25)	MeOH/H ₂ O (75/25)
Column	Acquity BEH Amide (1,0 x 100 mm, 1.7 μ m)	Acquity HSS T3 (1,0 x 100 mm, 1.8 μ m)
Temperature	30°C	40 °C
Injection Volume	0,5 μ L	
Trap	X Bridge C18 2,1 x 20 mm , 5 μ m	
Flow rate ISM	1mL/min of 10mM ammonium formate pH10.5 (NH ₄ OH)	
Cap Voltage	1,8kV	
Sampling cone	20 V	
Source Offset	80	
Temp Source	100 °C	
Temp désolvation	500°C	
Cone Gas	40 L/h	
Desolvation Gas	600 L/h	
Nebuliseur	6,5	



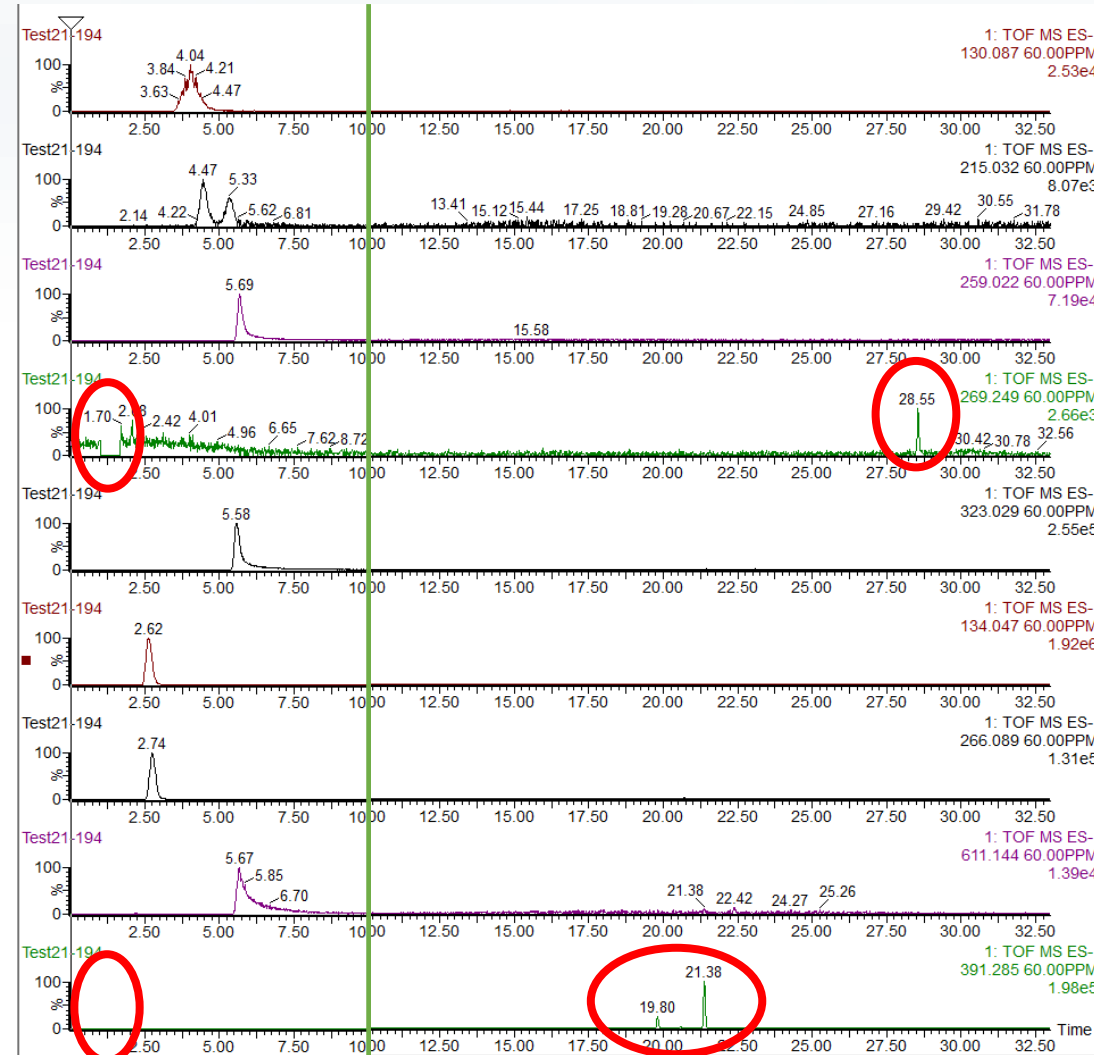
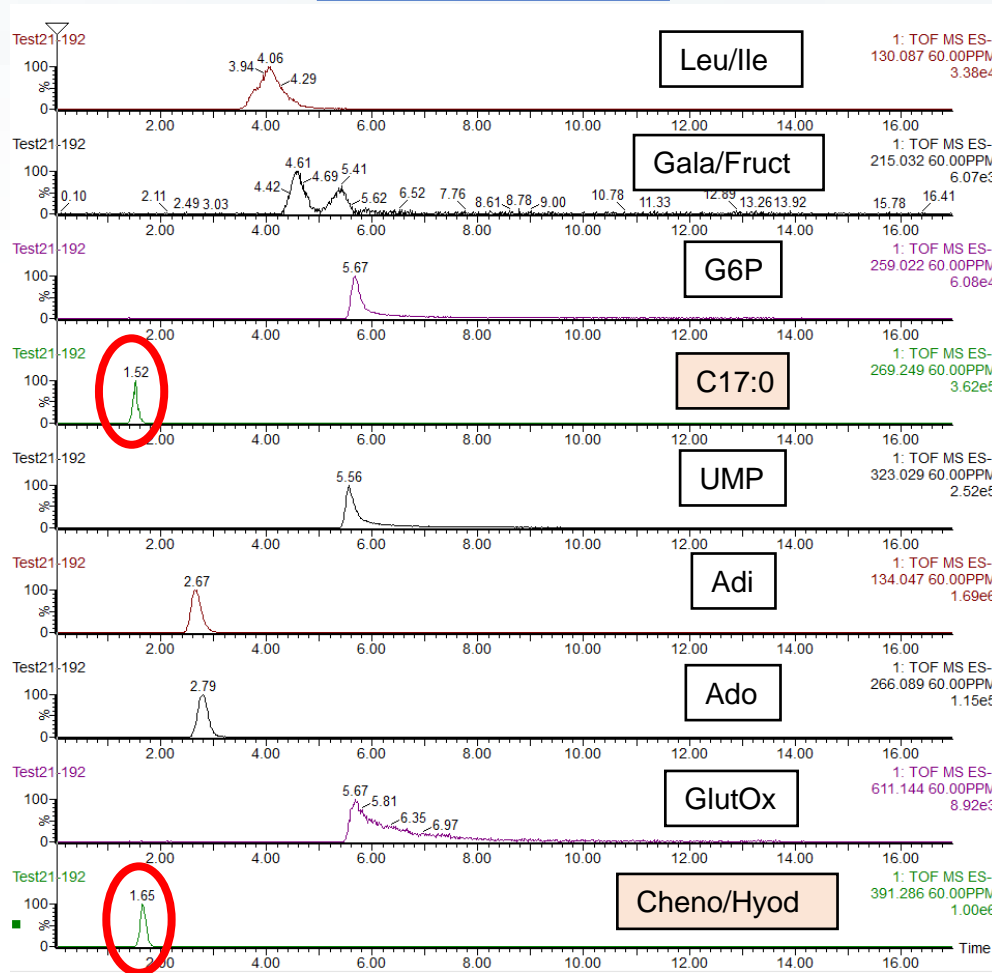
Waters Acquity UPLC I Class – QToF Synapt G2-Si HRMS

Electrospray source
 MS resolution ~15000 LeuEnk
 MS spectrum m/z 80 – 800
 Positive and negative ionisation

Development: standards mixture

2D μ HILIC- μ RP-MS

1D μ HILIC-MS



Leu/Ile

Gala/Fruct

G6P

C17:0

UMP

Adi

Ado

GlutOx

Cheno/Hyod

1D μ HILIC

2D μ RP

≠ physico-chemical properties, isomers
Cover whole elution range, co-elutions



NIST Plasma: extraction, annotation and identification



1D C18 LC-MS
⊕ & ⊖

1D μHILIC-MS
⊕ & ⊖

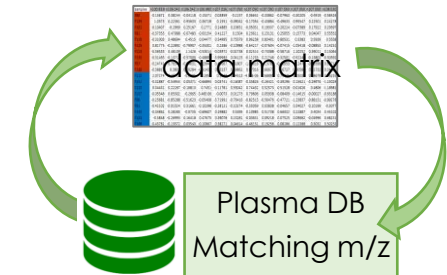
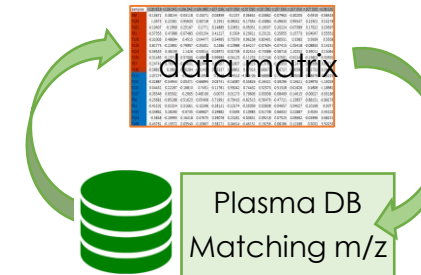
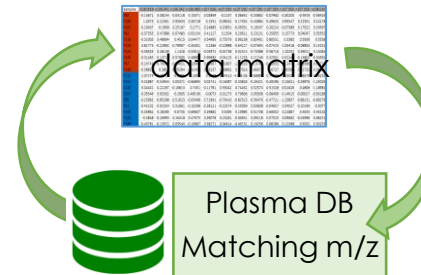
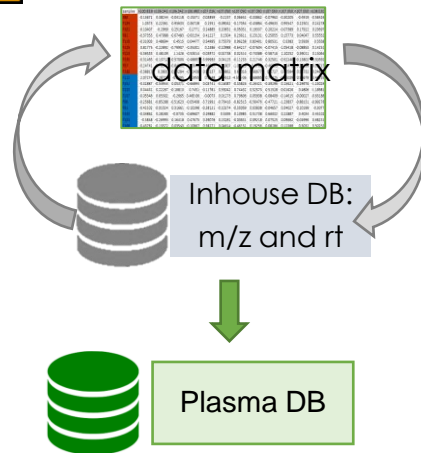
1D μRP-MS
⊕ & ⊖

2D μHILIC-μRP-MS
⊕ & ⊖

4
Wm
Workflow4metabolomics

Data extraction, alignment, blank filtration

<https://workflow4metabolomics.usegalaxy.fr>



Annotation and MS/MS identification



Final list of annotated metabolites in POS and NEG

Number of metabolites annotated for each method

1D C18 LC-MS → what's in the plasma?

Reference point for comparison



- Organic Acids
- Carnitines
- Vitamines
- Nucleic bases
- Peptides
- Amino Acids
- Carbohydrates
- Antioxydants
- Carboxylic Acids
- Bile Acids
- LysoPC LysoPE
- Free Fatty Acids

	1D C18 LC-MS	1D HILIC-MS	1D RP-MS	2D HILIC-RP-MS
POS	86	88	87	92
NEG	94	93	96	91
COM	73	72	68	65
TOTAL	253	253	251	248

What happended during the heart cutting? Which metabolites are lost?

1D C18-MS vs. 2D μ LC-MS: Where are they ?

n°	1D C18 LC-MS		DataBase v200130	2D μ HILIC- μ RP-MS					
	m/z	Rt (min) Hypersil	Compound	POS	m/z			NEG	m/z
107	130,0622	1,05	Creatine	POS	132,0771			NEG	130,0688
108	112,0516	0,96	Creatinine	POS	114,0668			NEG	112,0516
133	193,0497	8,47	Ferulic acid	POS	217,0457			NEG	193,0507
145	178,0506	4,74	Hippuric acid	POS	180,0655			NEG	178,0508
150	188,0713	11,55	Indole-3-propionic acid	POS	190,0867			NEG	188,0718
268	775,6760	18,17	Thyroxine	POS	777,6931			NEG	775,6785
115	314,2338	17,50	Decanoylcarnitine	POS	316,2493			NEG	314,2337

16	131,0716	6,90	2-hydroxycaproic acid	nd				NEG	131,0714	1,4	4,79	nd	
205	147,0657	1,90	Mevalonic acid	nd				NEG	147,0662	2,42	0,76	nd	
229	143,1074	18,79	Octanoic acid	nd				NEG	143,1077	1,06	9,22	nd	
238	192,0665	5,72	Phenylacetylglycine or 2-Methylhippuric acid	POS	194,0814	1,08	4,13	nd	NEG	192,0668	1,18	4,2	nd
275	111,0194	1,27	Uracil	POS	113,0347	2,21	0,8	nd	NEG	111,0197	2,22	0,8	nd

- 5/253 metabolites are lost during the heart-cutting
- ~40 metabolites have been annotated 2D μ LC-MS \rightarrow MS/MS confirm identification

Nd: not detected

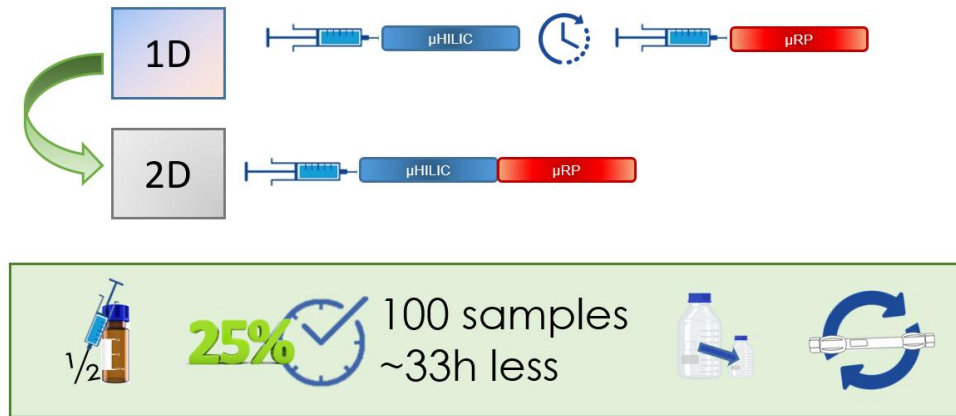


Conclusion

🔬 Developed 1D and 2D methods, repeatable, no carry-over:

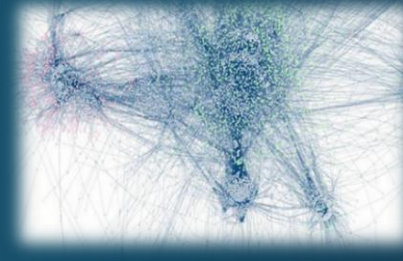
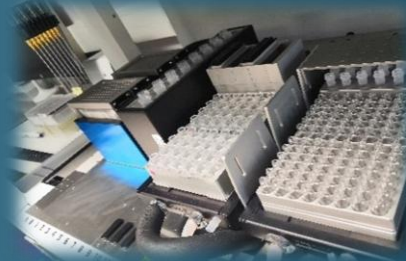
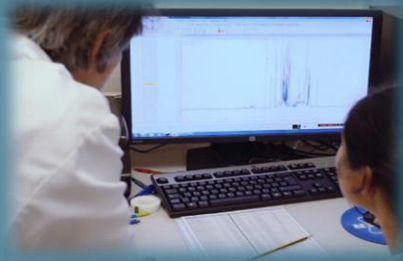
- Suitable for metabolomics

🔬 NIST plasma: detection of ~250 metabolites with consecutive μ HILIC- μ RP injections



🔬 Only 2% of identified metabolites are lost

- Realistically acceptable considering the improvement in analysis time and sample consumption
- ~40 metabolites \rightarrow MS/MS confirm identification




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Dermo-Cosmétique

Carine JACQUES
Hélène DUPLAN

Thank you for
your attention



Emilien JAMIN
Laurent DEBRAUWER

