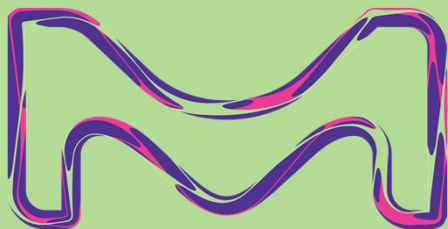


Maximizing Metabolite Detection

**Collaboration between
Aristotle University and Merck**

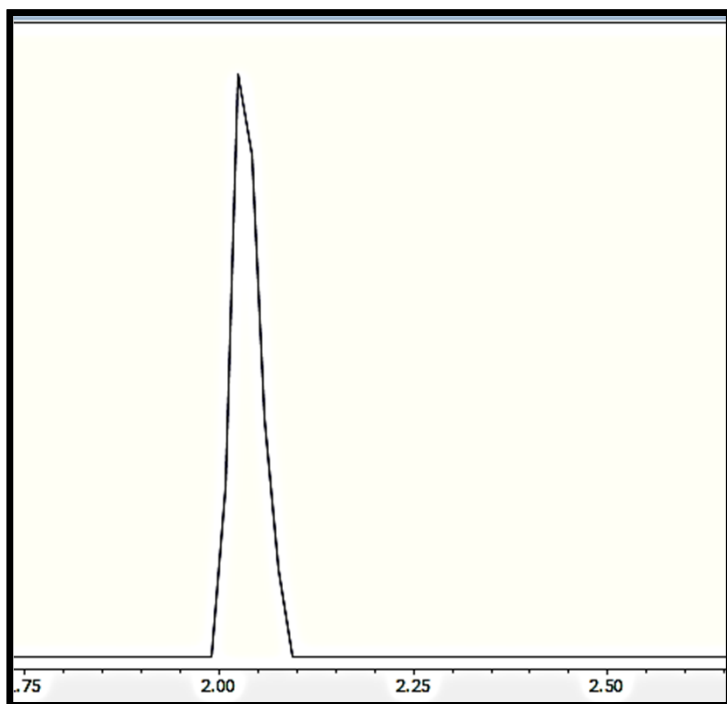
Shari Spector, PhD
Head of Biochemicals Innovation
March 16, 2021



MERCK

Challenges in Metabolomics

Metabolite identification with LC-MS-based methods



Ensuring accurate metabolite ID

- Reference compounds needed to confirm retention time, molecular mass, and fragmentation patterns

Maximizing the number of detected metabolites

- Column chemistry
- Chromatography conditions
- Mass spectrometry instrumentation
 - Resolution
 - Data acquisition settings
 - Ionization mode

Establishing retention time and MS behavior

Mass Spectrometry Metabolite Library of Standards



Mass Spectrometry Metabolite Library of Standards (MSMLS)

- Seven plates comprising:
 - Carboxylic acids and amino acids
 - Biogenic amines and polyamines
 - Nucleotides, coenzymes and vitamins
 - Mono- and disaccharides
 - Fatty acids, lipids, steroids and hormones

BACSMLS – Bile acid, carnitine and sterol library

FAMLS – Fatty acid library

OAMLS – Organic acid library

TOTAL - 922 metabolites

Detection of Metabolites

Chromatography and High-Resolution Mass Spectrometry

Separation Methods

Reversed phase columns:

Supelco Ascentis® Express AQ-C18

Supelco Ascentis® Express AQ-C8

Supelco Ascentis® Express AQ-RP-Amide

HILIC columns:

Supelco Ascentis® Express OH5

SeQuant® zHILIC

Detection Methods

timsTOF Mass Spectrometer (Bruker)

Trapped Ion Mobility Spectroscopy

Positive and negative ionization modes



Dimitra Diamantidou, Dr. Katerina Kademoglou, and Eleftheria Boutou

LC-MS analysis

What conditions enable maximum detection of the 922 metabolites?

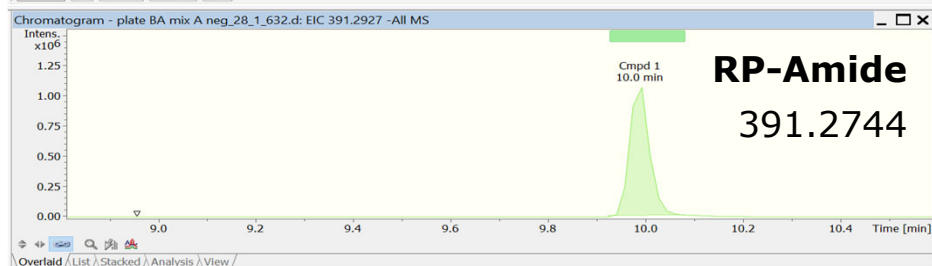
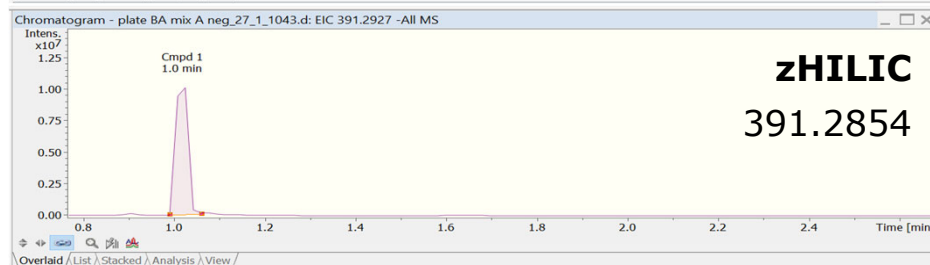
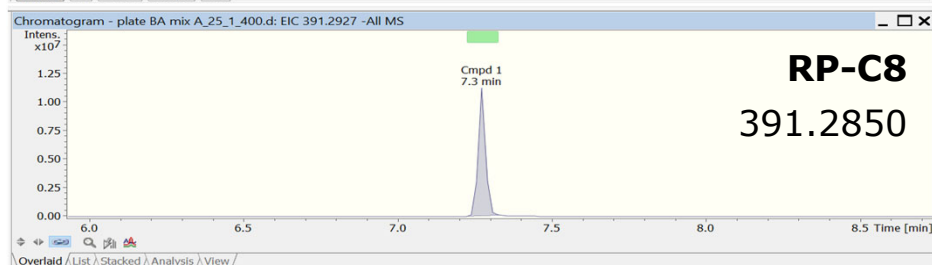
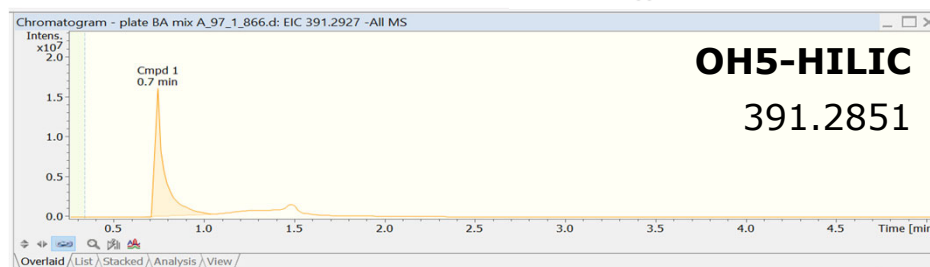
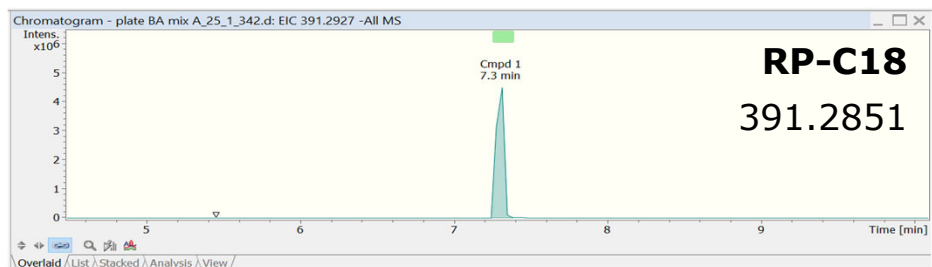
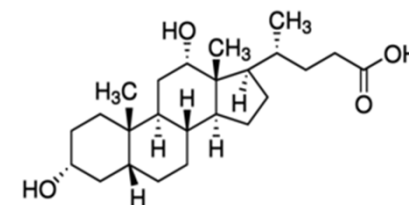
Plate	Reverse Phase Separations						HILIC Separations			
	RP-C18		RP-C8		RP-amide		OH5		zHILIC	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Plates 1-5, 7	+	+	+	+	+	+	+	+	+	+
Plate 6: Lipids	+	+	+	+	+	+	+	+	+	+
Plate 6: Sugars	NA	NA	NA	NA	NA	NA	+	+	+	+
BACSMLS	NA	+	carnitines	+	NA	+	NA	+	NA	+
FAMLS	NA	+	NA	+	NA	+	NA	+	NA	+
OAMLS	NA	+	NA	+	NA	+	NA	+	NA	+

NA – not analyzed

TOTAL: 7726 chromatograms!

Chromatographic separations – Bile Acids

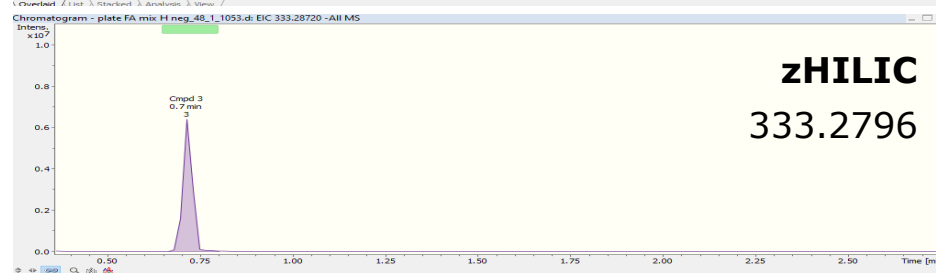
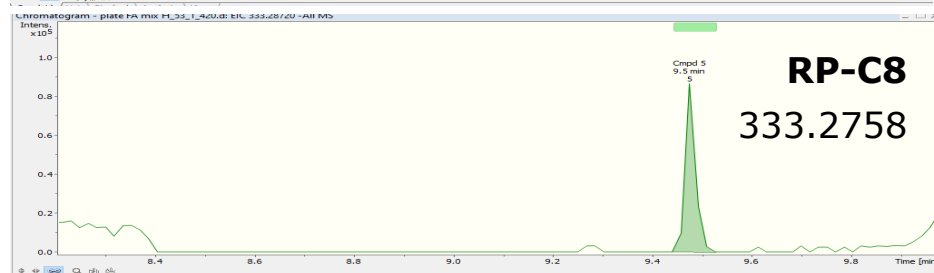
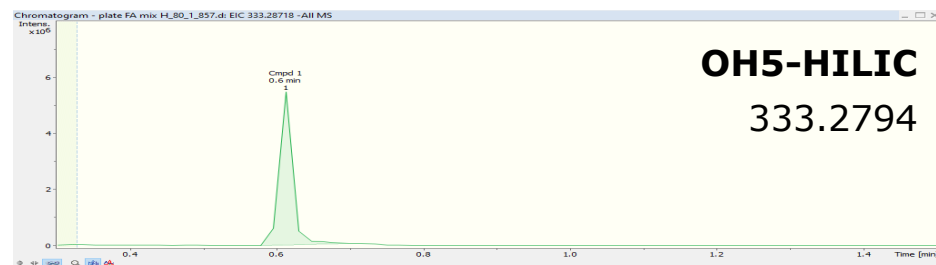
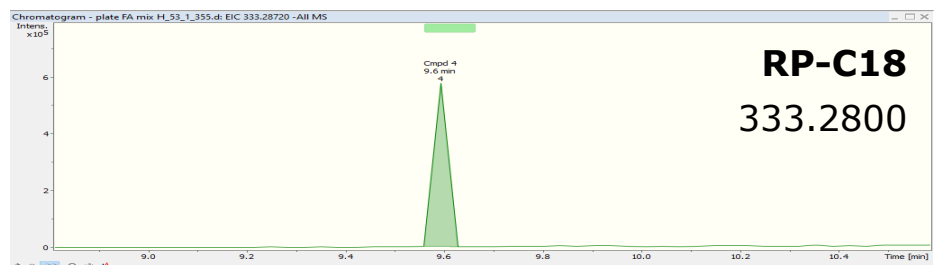
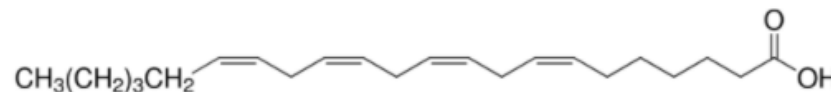
Deoxycholic acid



BACSMLS, well A4
 Monoisotopic mass [M-H]⁻: 391.2927
 Negative ionization mode

Chromatographic separations – Fatty acids

Docosatetraenoic acid

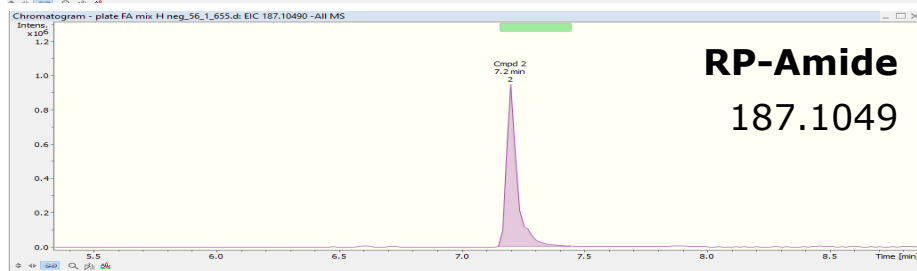
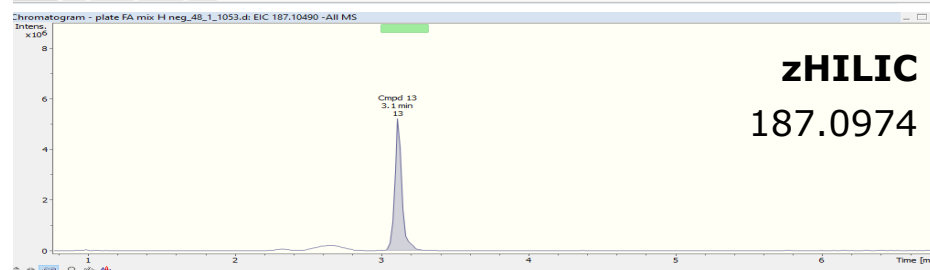
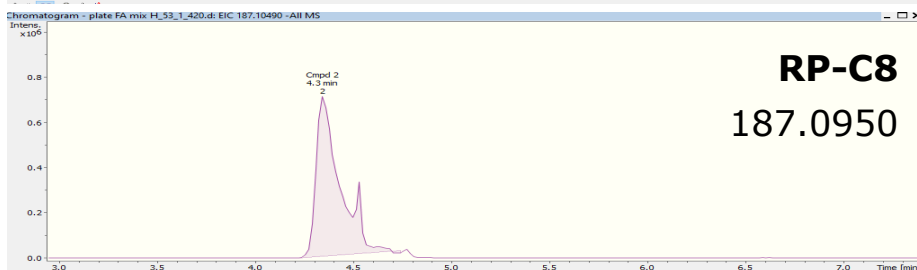
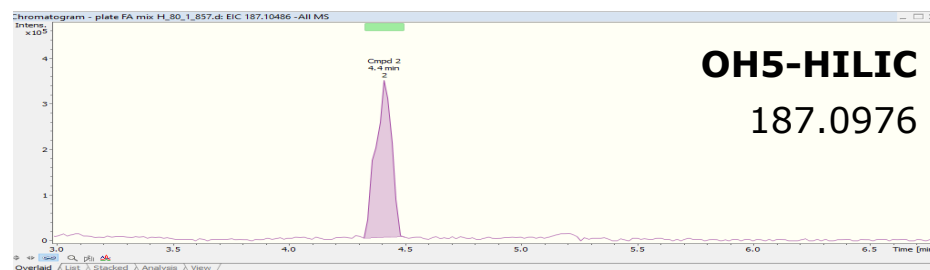
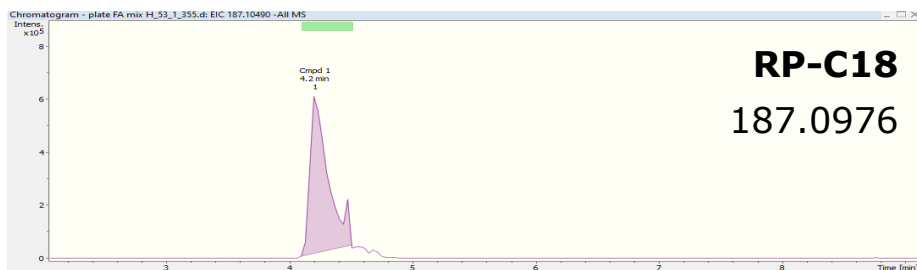
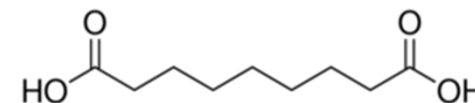


RP-Amide
Not Detected

FAMLS, well H10
 Monoisotopic mass [M-H]⁻: 333.2872
 Negative ionization mode

Chromatographic separations – Fatty Acids

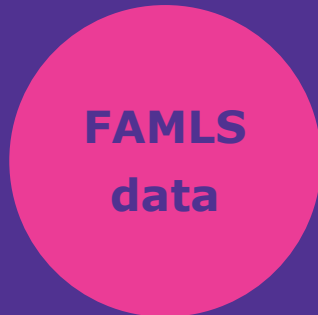
Azaleic Acid



FAMLS, well H5
Monoisotopic mass [M-H]⁻: 187.1049
Negative ionization mode

LC-MS analysis

- timsTOF data collection:
 - 992 metabolites
 - 5 columns; +/- ionization modes
 - 7726 LC-MS chromatograms
- Data analysis: 77% complete, with 5912 analyses complete; 1814 remain
- Assessing
 - **Detection by column**
 - Detection by metabolite class
 - Chromatographic performance (peak shape, etc.)



1	Detected
0	Not Detected
WP	Work in Progress

	C18	C8	amide	OH5	ZHILIC
Phenacetic acid	1	1	1	1	1
Phenylacetic acid	1	1	1	1	1
15-Methylglutamic acid	1	1	1	1	1
2-Hydroxyglutamic acid	1	1	1	1	1
Isopropionic acid	1	1	1	1	1
16-Methylglutamic acid	1	1	1	1	1
2-Hydroxybutyric acid	1	1	1	1	1
Isobutyric acid [15-Methylglutamic acid]	1	1	1	1	1
17-Methylglutamic acid	1	1	1	1	1
trans-11-Cinnamic acid	1	1	1	1	1
all-cis-4',7',10',13',16'-Dioxopropionic acid	1	1	1	1	1
Hydroxyacetic acid	1	1	1	1	1
Linoleic acid	1	1	1	1	1
oleic acid	1	1	1	1	1
cis-8,11,14-Eicosatrienoic acid	1	1	1	1	1
cis-11,14-Eicosadienoic acid	1	1	1	1	1
Palmitic acid	1	1	1	1	1
Heptadecanoic acid	1	1	1	1	1
Docosanoic acid [Dodecanoic acid]	1	1	1	1	1
Triacontanoic acid	1	1	1	1	1
Hexanoic acid	1	1	1	1	1
Linoleic acid	1	1	1	1	1
Propanoic acid	1	1	1	1	1
Octanoic acid	1	1	1	1	1
Valeric acid	1	1	1	1	1
cis-5-Dodecanoic acid	1	1	1	1	1
Undecanoic acid	1	1	1	1	1
Tridecanoic acid [Hydroquinic acid]	1	1	1	1	1
Myristic acid	1	1	1	1	1
15-Methylpalmitic acid	1	1	1	1	1
Stearic acid	1	1	1	1	1
cis-5,8,11,14,17-Eicosapentaenoic acid	1	1	1	1	1
cis-11-Eicosenoic acid	1	1	1	1	1
Arachidonic acid	1	1	1	1	1
Dodecapentaenoic acid [cis-7,10,13,16,19]	1	1	1	1	1
Myristic acid	1	1	1	1	1
2-Methylglutamic acid	1	1	1	1	1
2-Methyl-4-pyruvic acid	1	1	1	1	1
2-Hydroxy-2-methylglutamic acid	1	1	1	1	1
2-Ethyl-2-hydroxyglutamic acid	1	1	1	1	1
Isopropylmalonic acid	1	1	1	1	1
2-Methylsuccinic acid	1	1	1	1	1
3,3-Dimethylglutamic acid	1	1	1	1	1
cis-5-Dodecanoic acid	1	1	1	1	1
17-Oxidodecanoic acid	1	1	1	1	1
12-Methyltridecanoic acid	1	1	1	1	1
Coumaric acid [12,14E]-Linoleic acid [Dodecanoic acid]	1	1	1	1	1
trans-2-Methyl-2-hydroxyacetic acid	1	1	1	1	1
2-Hydroxyacetic acid	1	1	1	1	1
18-Hydroxyacetic acid	1	1	1	1	1
Lipoic acid, reduced	1	1	1	1	1
Tetanic acid	1	1	1	1	1
Propanoic acid	1	1	1	1	1
Heptadecanoic acid	1	1	1	1	1
Hexadecanoic acid	1	1	1	1	1
Arachidonic acid	1	1	1	1	1
[12], [12E], [12E]-Eicosatrienoic acid	1	1	1	1	1
Dodecahexanoic acid	1	1	1	1	1
[1]-5-Hydroxyundecanoic acid	1	1	1	1	1
5-Dodecanoic acid	1	1	1	1	1
Dodecanoic acid	1	1	1	1	1
18:1HAD [18-hydroxy-2-dodecanoic acid]	1	1	1	1	1
18-Hydroxydodecanoic acid	1	1	1	1	1
Palmitic acid	1	1	1	1	1
Xenobiotic acid	1	1	1	1	1
Linoleic acid	1	1	1	1	1
P-Hydroxylinoleic acid	1	1	1	1	1
cis-18-Hydroxyoctadecanoic acid	1	1	1	1	1
Tetrahydrocannabinolic acid	1	1	1	1	1
Octanoic acid	1	1	1	1	1
Hexanoic acid	1	1	1	1	1
Hydroxyacetic acid	1	1	1	1	1
[1]-12-Hydroxyundecanoic acid	1	1	1	1	1
Octanoic acid	1	1	1	1	1
Linoleic acid	1	1	1	1	1
Palmitic acid	1	1	1	1	1
Stearic acid	1	1	1	1	1
oleic acid	1	1	1	1	1
Stearic acid	1	1	1	1	1
192,162-Dodecahydroxystyrene acid	1	1	1	1	1
Hexanoic acid	1	1	1	1	1
Isobutyric acid	1	1	1	1	1
trans-3-Hydroxyacetic acid	1	1	1	1	1
Hexanoic acid	1	1	1	1	1
Heptanoic acid	1	1	1	1	1
[12], [12E], [12E]-Eicosatrienoic acid	1	1	1	1	1
trans-11-Cinnamic acid	1	1	1	1	1
Stearic acid	1	1	1	1	1
oleic acid	1	1	1	1	1
cis-7,10,13,16,19-Dodecapentaenoic acid	1	1	1	1	1
Dodecahexanoic acid	1	1	1	1	1
Ervicic acid	1	1	1	1	1



LC-MS analysis

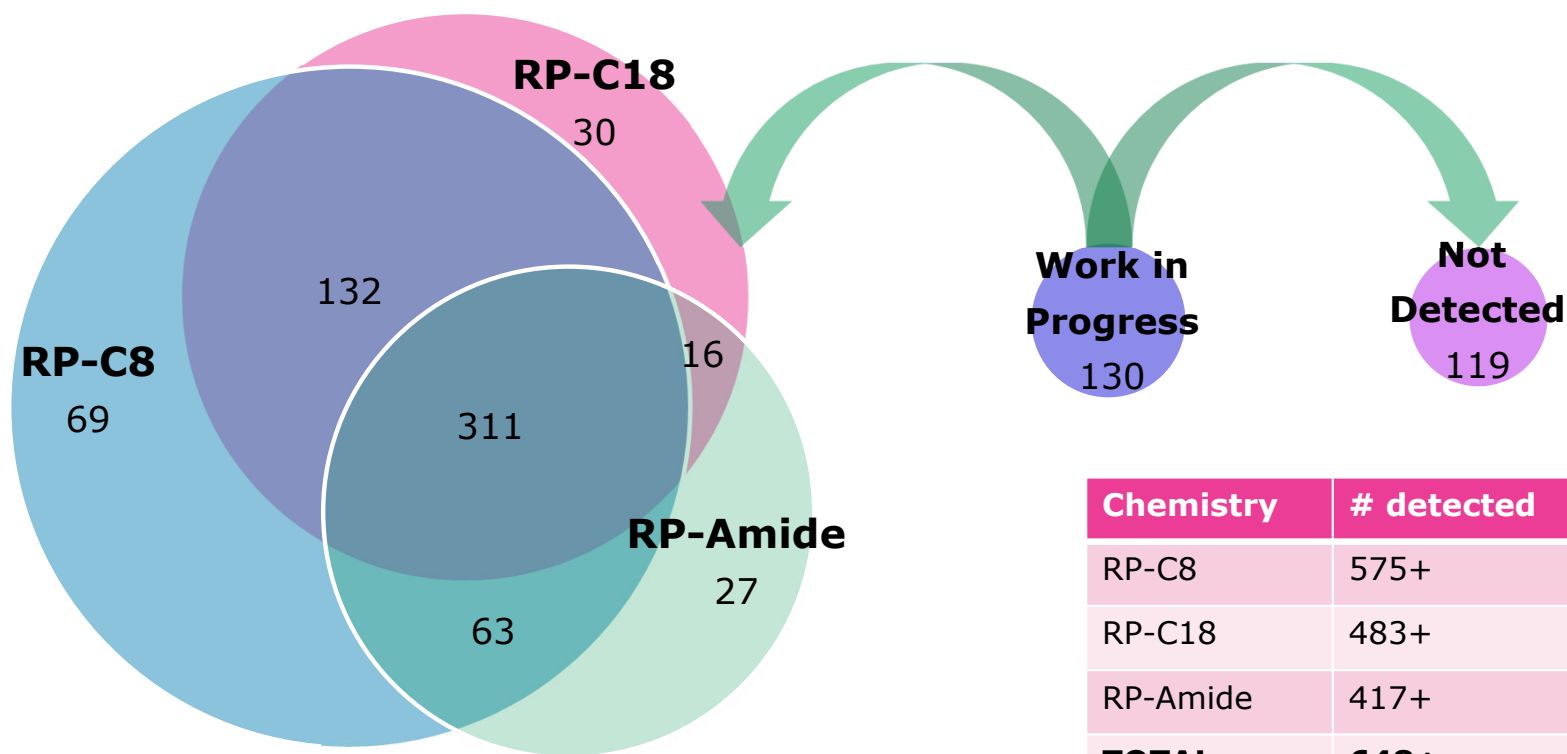
Which columns and ionization modes enable maximum detection?

Plate	Reverse Phase Separations						HILIC Separations			
	RP-C18		RP-C8		RP-amide		OH5		zHILIC	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Detected	188	432	311	444	230	312	205	345	231	396
	489		575		417		430		477	
Work in Progress	183		97		109		192		332	
Not detected	225		225		371		300		113	
Total analysis	897		897		897		922		922	
Not analyzed	25		25		25		-		-	

Analysis completed for 5912 / 7726

Metabolite Coverage – Reverse Phase Chromatography

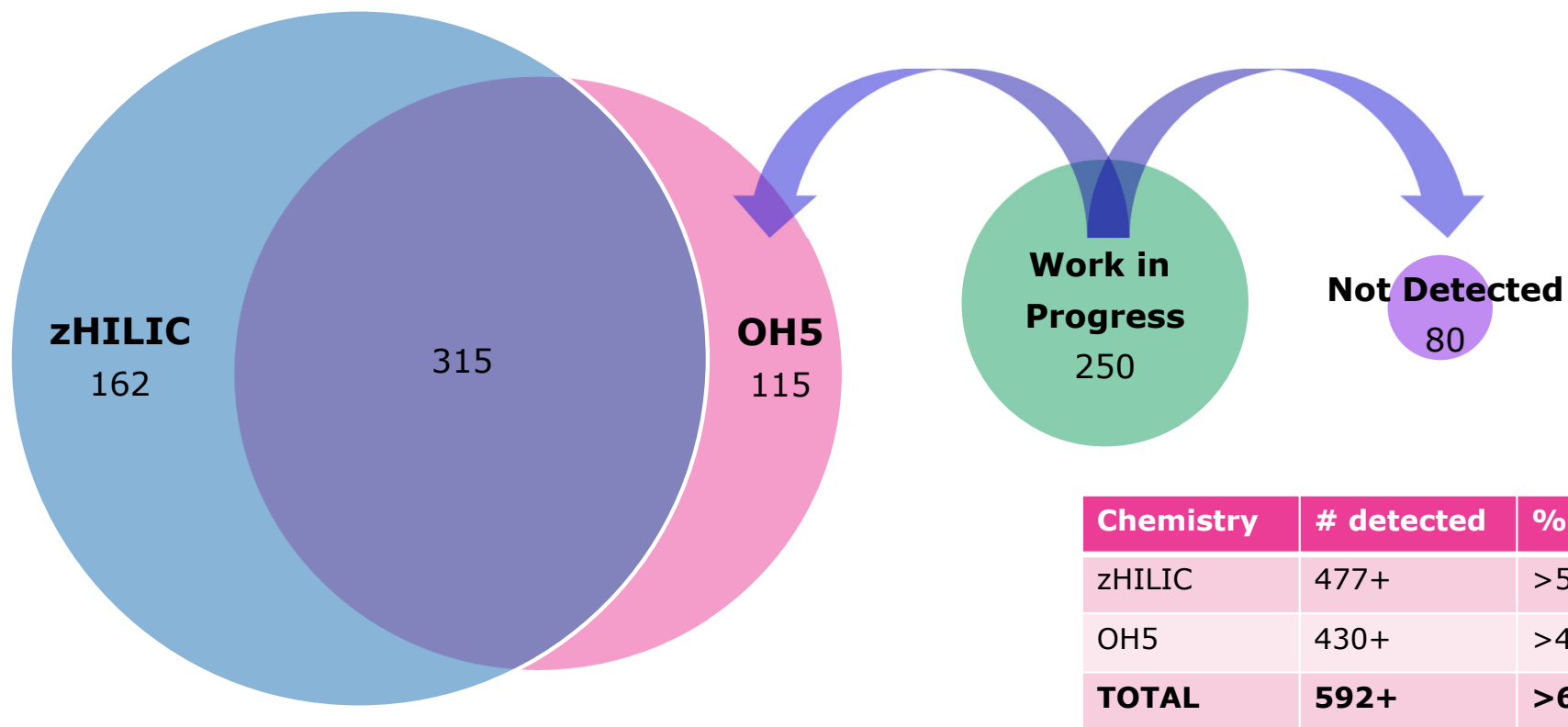
648+ metabolites detected by RP-LC-MS



Chemistry	# detected	% detected
RP-C8	575+	>62%
RP-C18	483+	>52%
RP-Amide	417+	>45%
TOTAL	648+	>70%

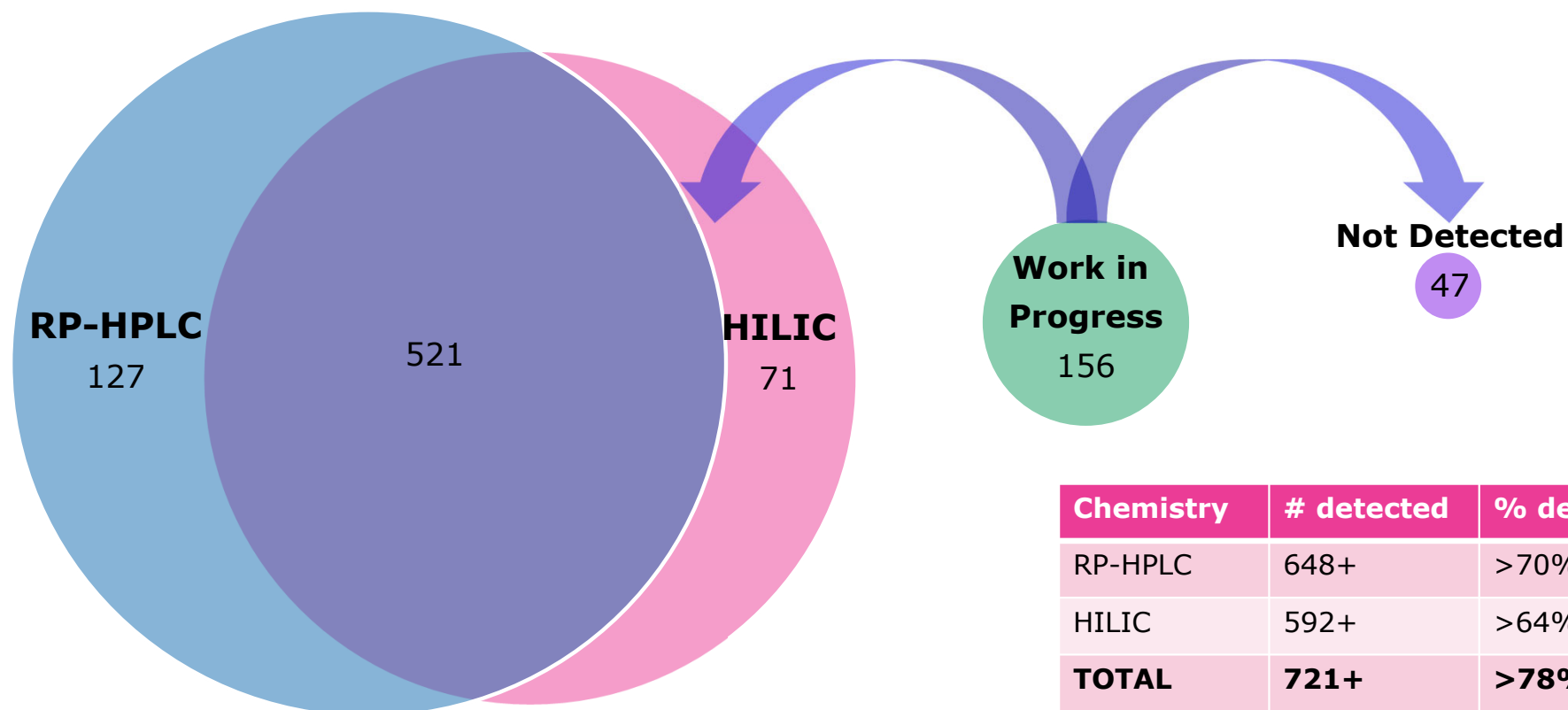
Metabolite Coverage – HILIC Chromatography

592+ metabolites detected by HILIC-LC-MS



Metabolite coverage

721+ metabolites detected across all conditions



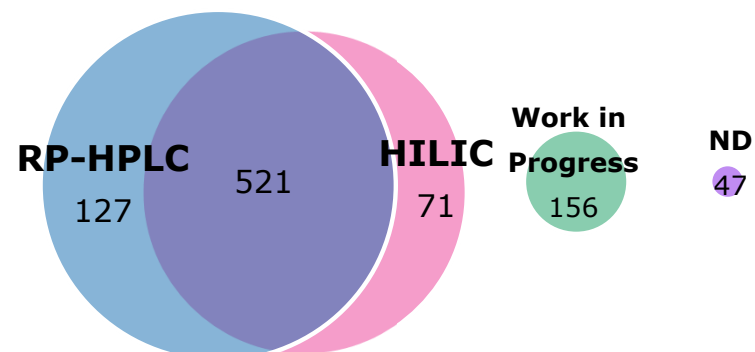
Interim conclusions

Analysis of 992 metabolites x 10 LC-timsTOF-MS experiments

- MSMLS: Masses range from ~70 – 1500 Da
 - 721 metabolites detected (78% of expanded MSMLS library)
 - 47 metabolites not detected under any of the conditions tested (5%)
 - 154 additional metabolites to be analyzed (17% of library)
- No single analysis mode will detect all metabolites
- First detailed analysis of OA / BA / FA

Ongoing work

- Assess column performance across metabolites
- Recommend analytical conditions by class of metabolites
- Obtain CCS values for each metabolite
- Retention prediction; comparison with experimental data



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Thank you

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<https://www.sigmaaldrich.com/life-science/metabolomics.html>

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