Development of an ultra high sensitive bioanalytical method with 2D-microUHPLC

Pictured above: HIV absorption

Liesbeth Vereyken, November 19, 2015 | Discovery and Exploratory Bioanalysis – PDM – Discovery Sciences

janssen

Outline

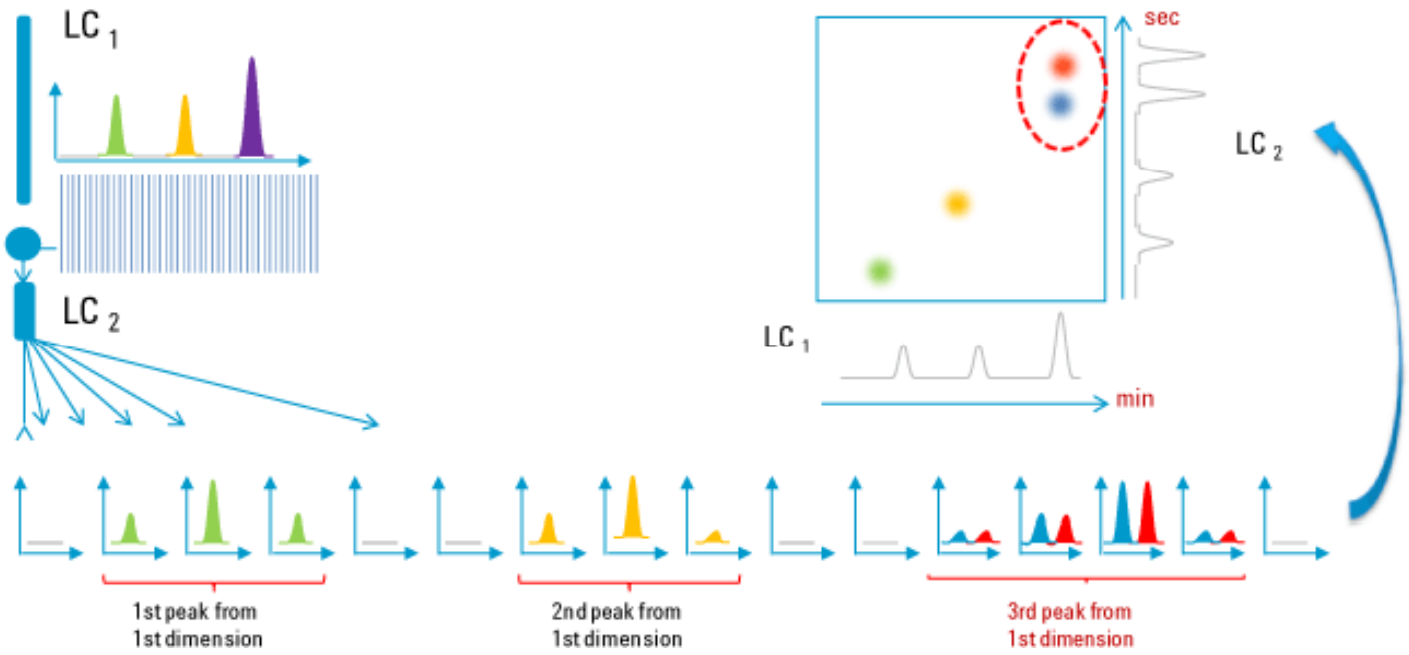
- 2D LC concept and our aim
- Our 2D micro-system and how it works
- Preliminary work: direct column injections, heart cut determination,…
- Practical considerations during optimization
- Results
- Conclusion

Outline

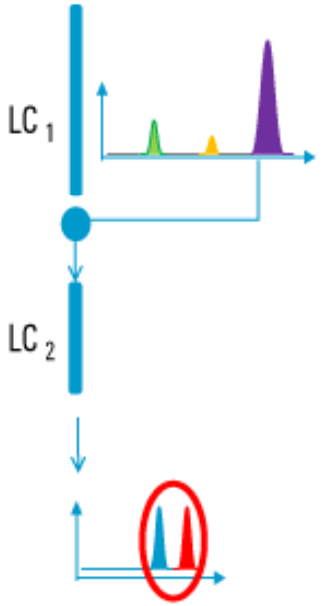
- **2D LC concept and our aim**
- Our 2D micro-system and how it works
- Preliminary work: direct column injections, heart cut determination,…
- Practical considerations during optimization
- Results
- Conclusion

Two dimensional LC: concept

Comprehensive 2D-LC



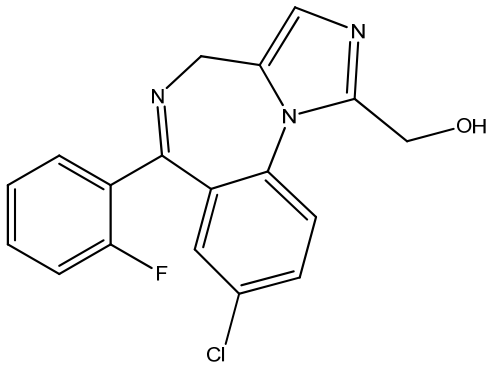
Heart-cutting 2D-LC



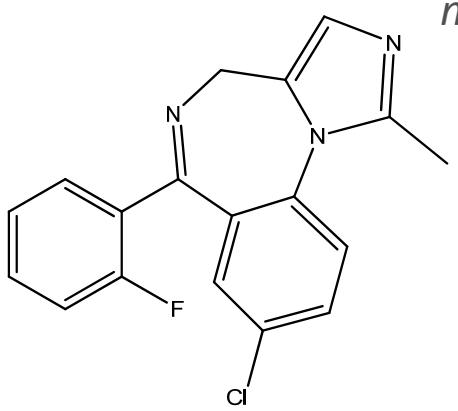
Two dimensional LC: our aim

- More sensitivity for a lower LLOQ with limited sample prep: sample availability/lower dosing
- Optimizing a generic heart-cut 2D micro-LC with ionKey
- Overcome ion suppression/background reduction with large volume injections - 50µl
- Understanding practical limitations of the system

1'-OH-midazolam
m/z 342



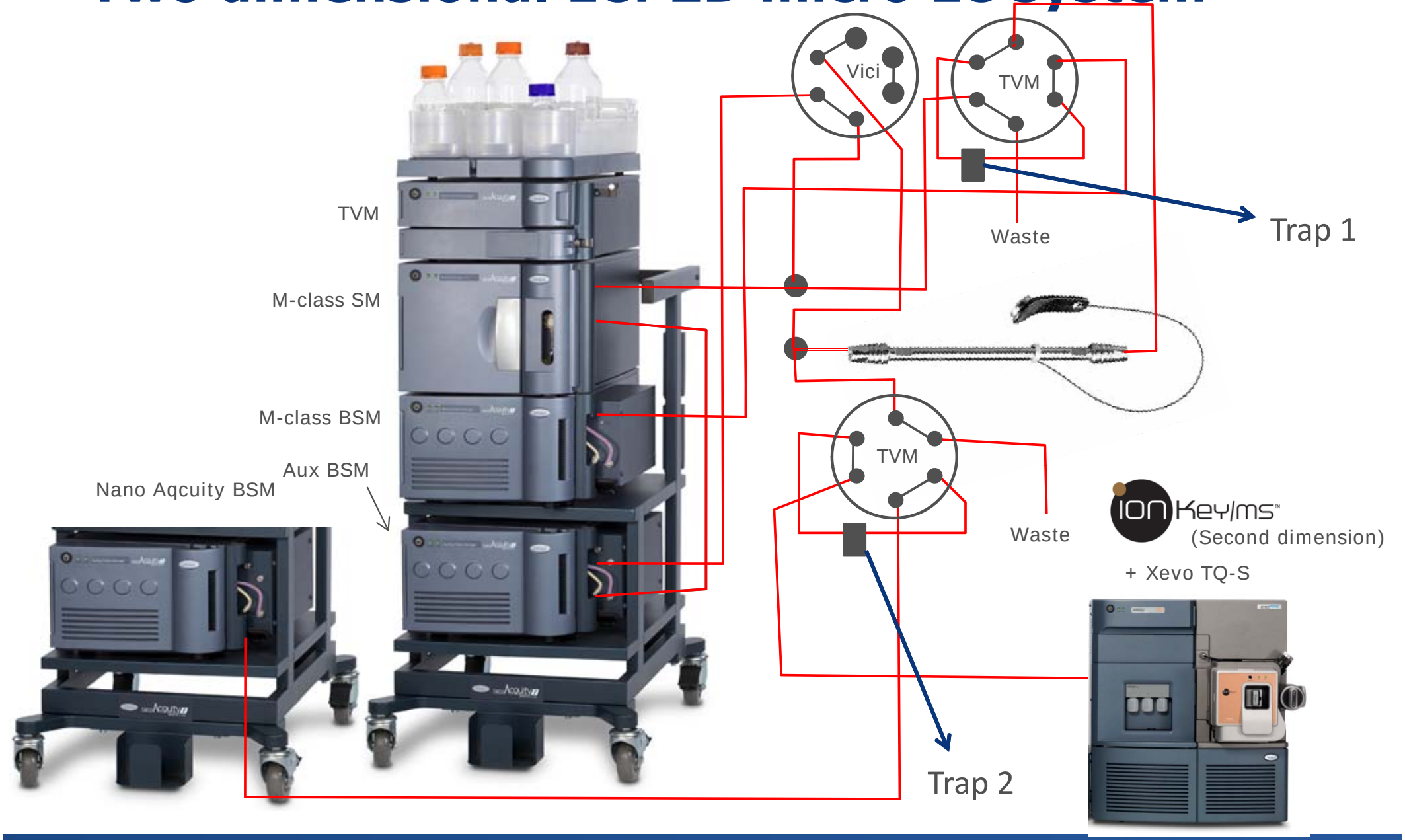
midazolam
m/z 326

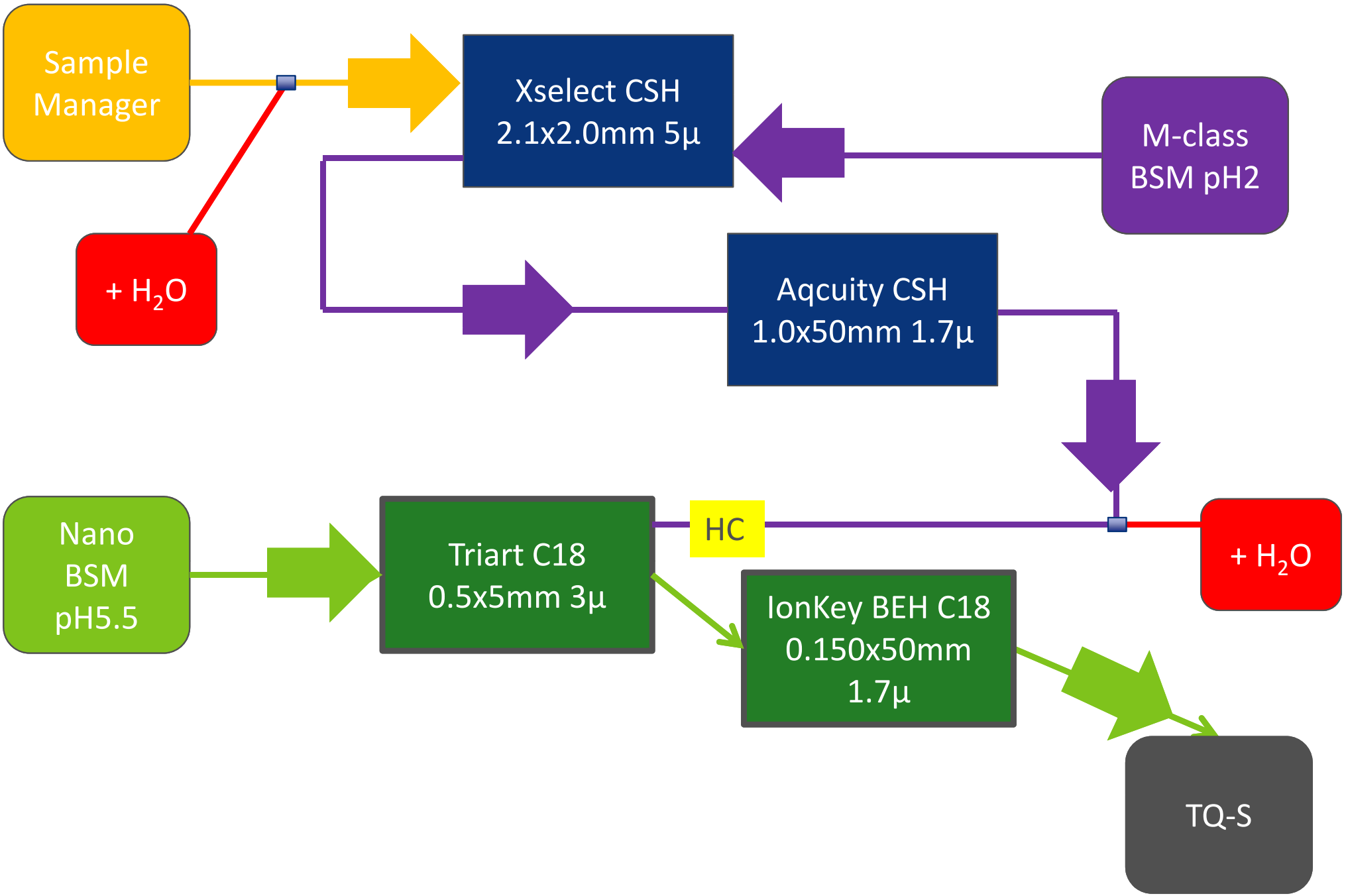


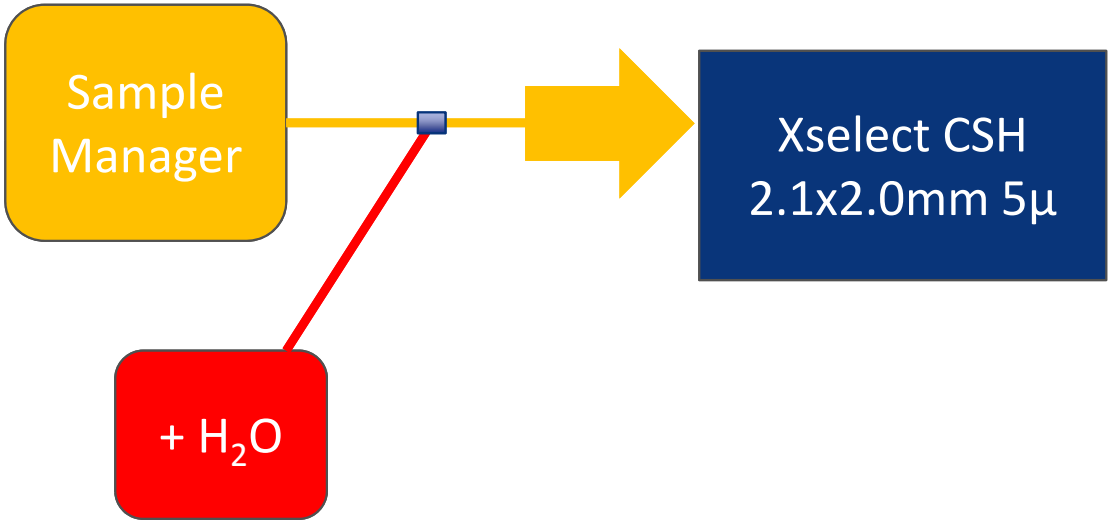
Outline

- 2D LC concept and our aim
- **Our 2D micro-system and how it works**
- Preliminary work: direct column injections, heart cut determination,…
- Practical considerations during optimization
- Results
- Conclusion

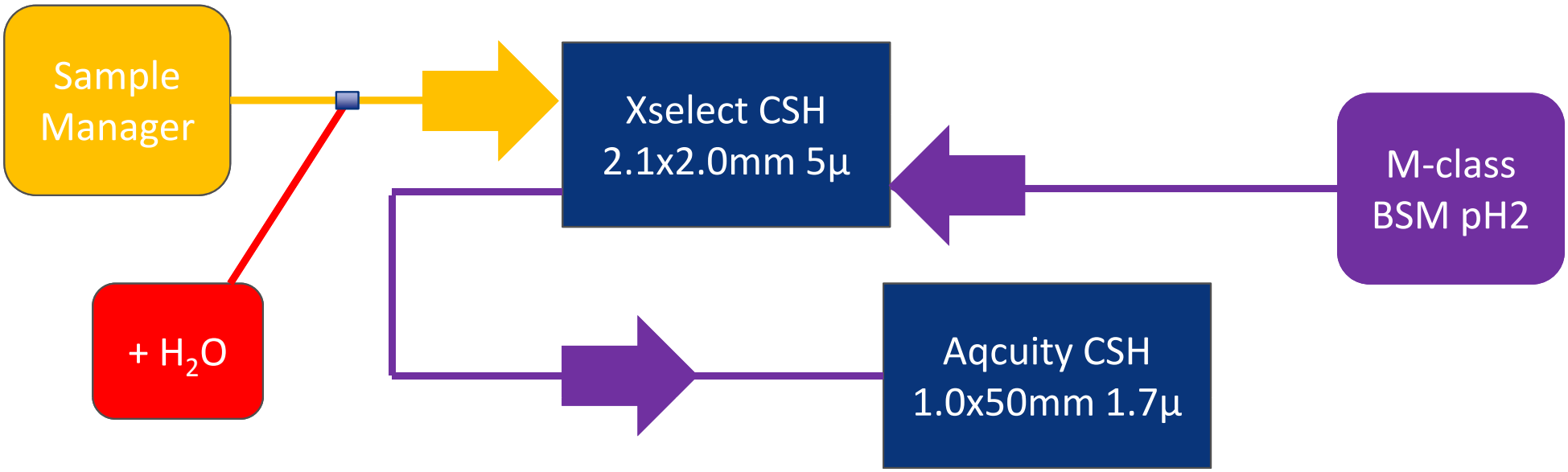
Two dimensional LC: 2D micro LC system



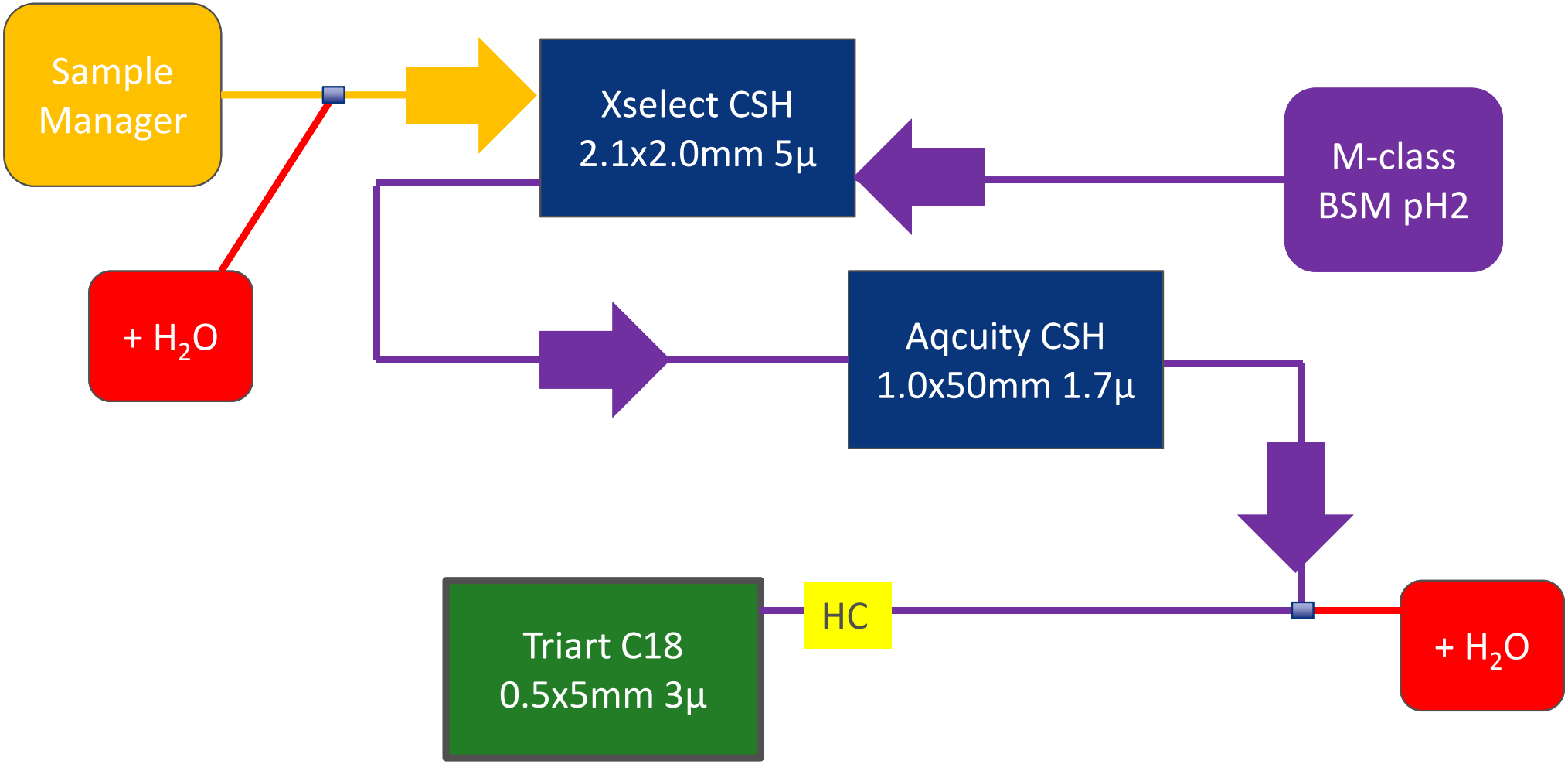

Sample Manager
+ H_2O
Xselect CSH 2.1x2.0mm 5µ
M-class BSM pH2
Aqcuity CSH 1.0x50mm 1.7µ
Nano BSM pH5.5
Triart C18 0.5x5mm 3µ
HC
+ H_2O
IonKey BEH C18 0.150x50mm 1.7µ
TQ-S



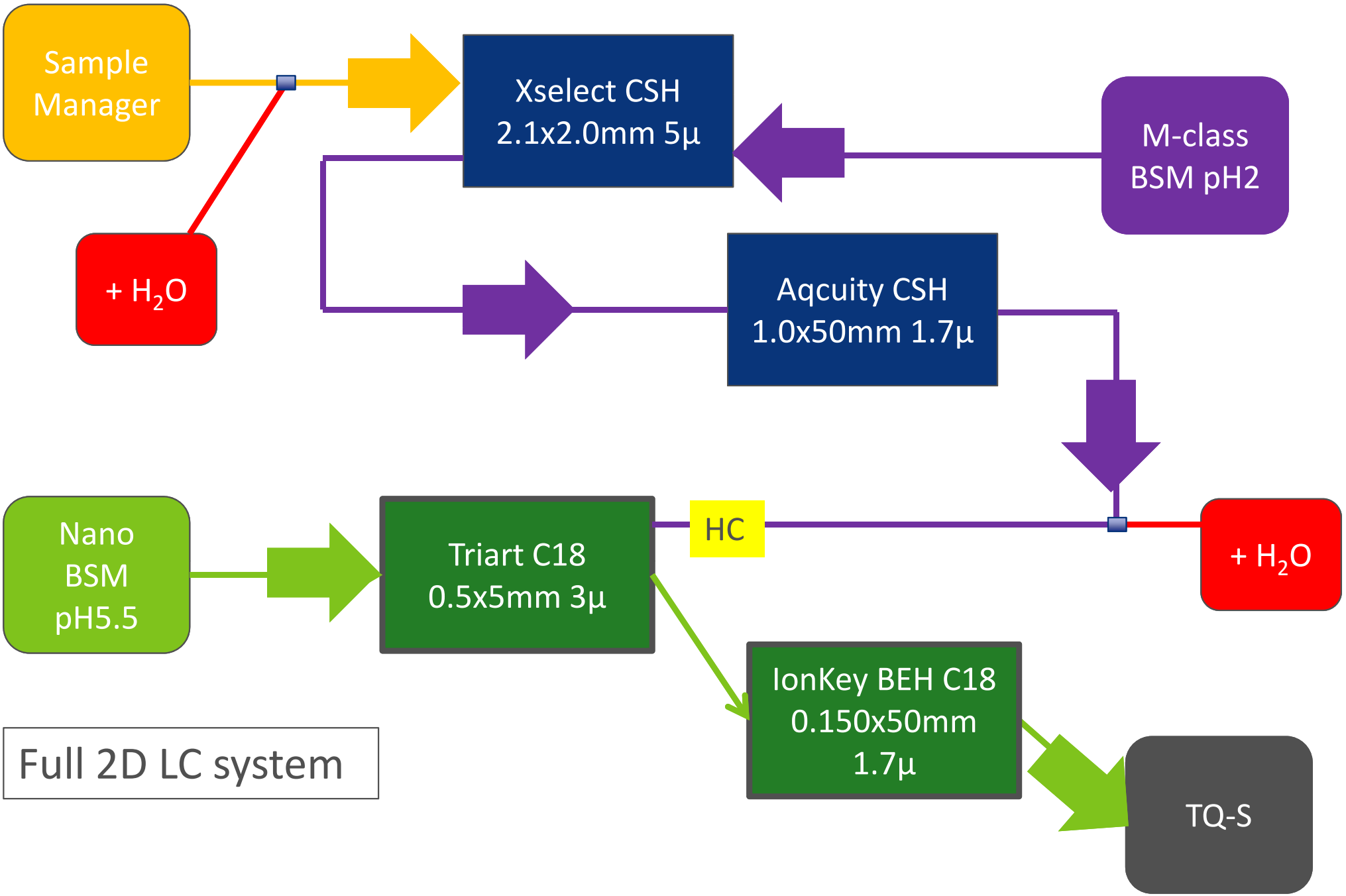
First dimension TRAP



First dimension
TRAP + ELUTE



Second dimension
HEART CUT



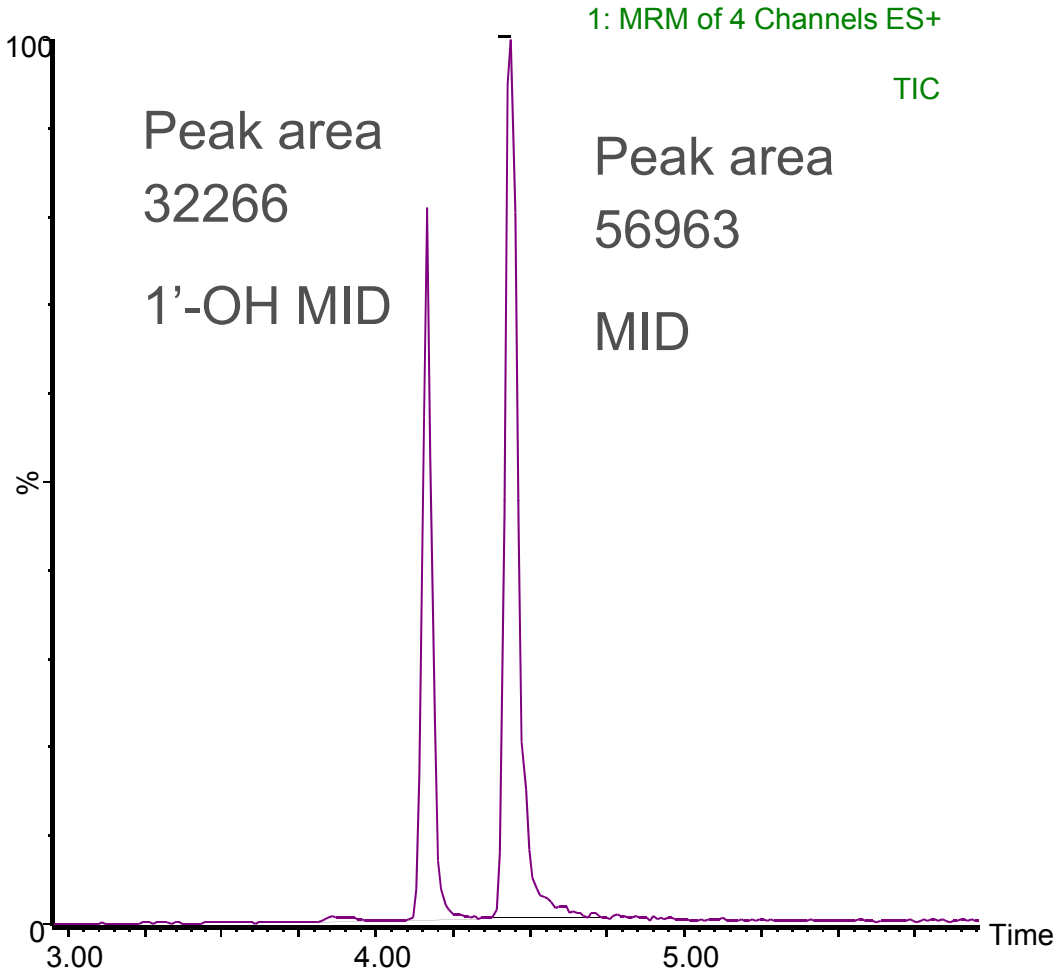
Full 2D LC system

Outline

- 2D LC concept and our aim
- Our 2D micro-system and how it works
- **Preliminary work: direct column injections, heart cut determination,…**
- Practical considerations during optimization
- Results
- Conclusion

Preliminary work: IonKey/MS

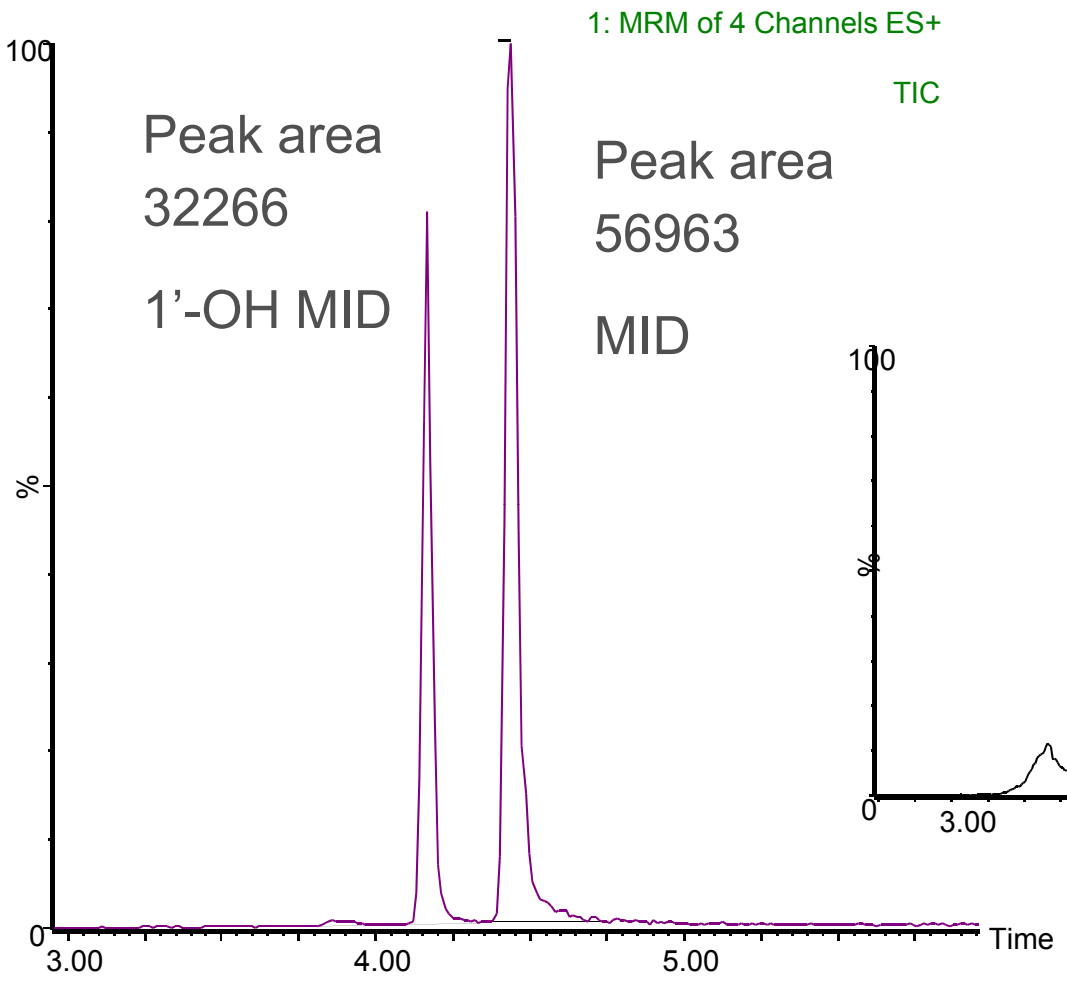
0.2 µl injection 2µl/min flow

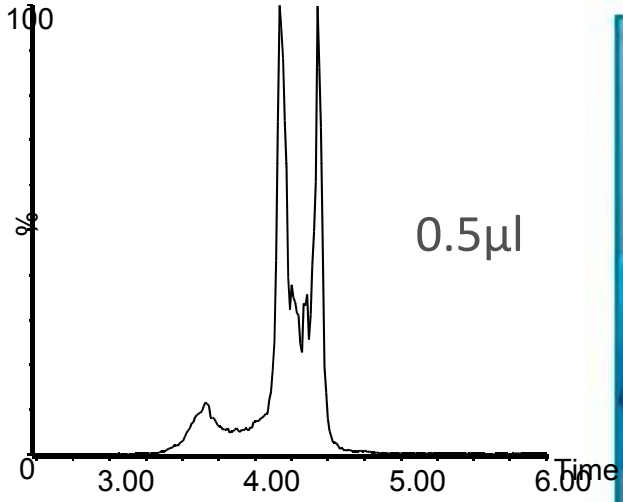


IonKey BEH C18 0.150x50mm 1.7µ

Preliminary work: IonKey/MS

0.2 µl injection 2µl/min flow





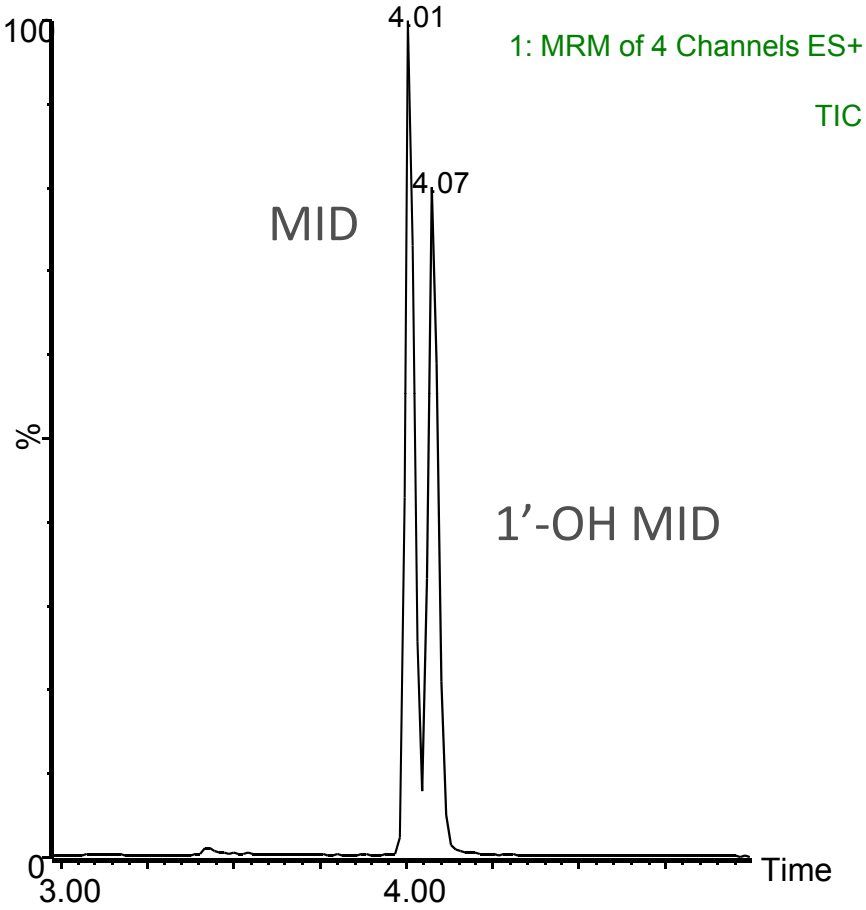
0.5µl

IonKey BEH C18 0.150x50mm 1.7µ

Preliminary work: first dimension

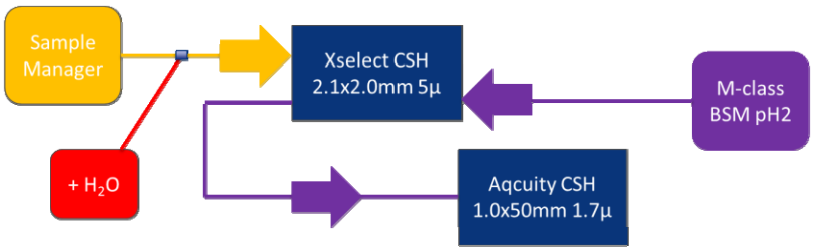
Trap → Xselect CSH phenyl-hexyl 5µ 2.1x20mm

Elute → Acquity CSH phenyl-hexyl 1.7µ 1.0x50mm



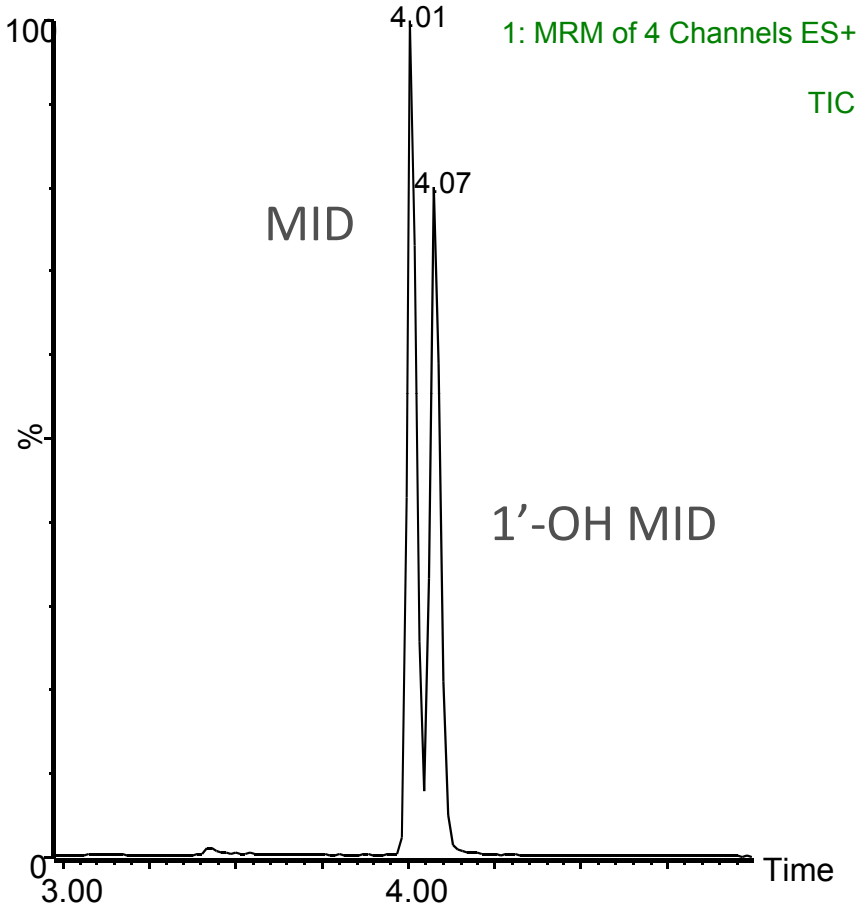
Trap1: isocratic
Aux pump A: H_2O flowrate 400µl/min
Aux pump B: H_2O/ACN/IPA (40/40/20)
flowrate 100µl/min

Elute: gradient flowrate 50µl/min
m-class pump A: 0.1% Formic acid
m-class pump B: Acetonitrile

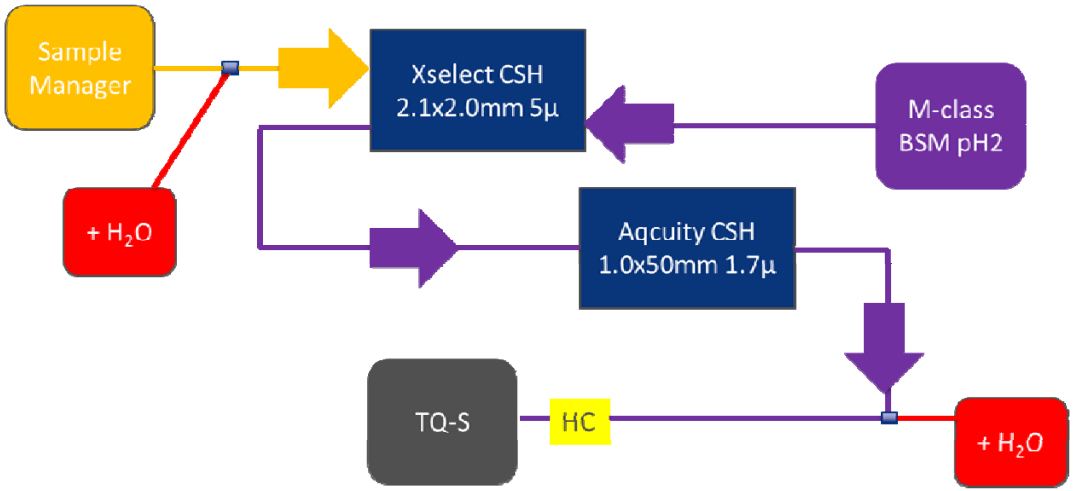


Preliminary work: determining heart-cut window

Peakwidth 0.2 min, minimal window for hc

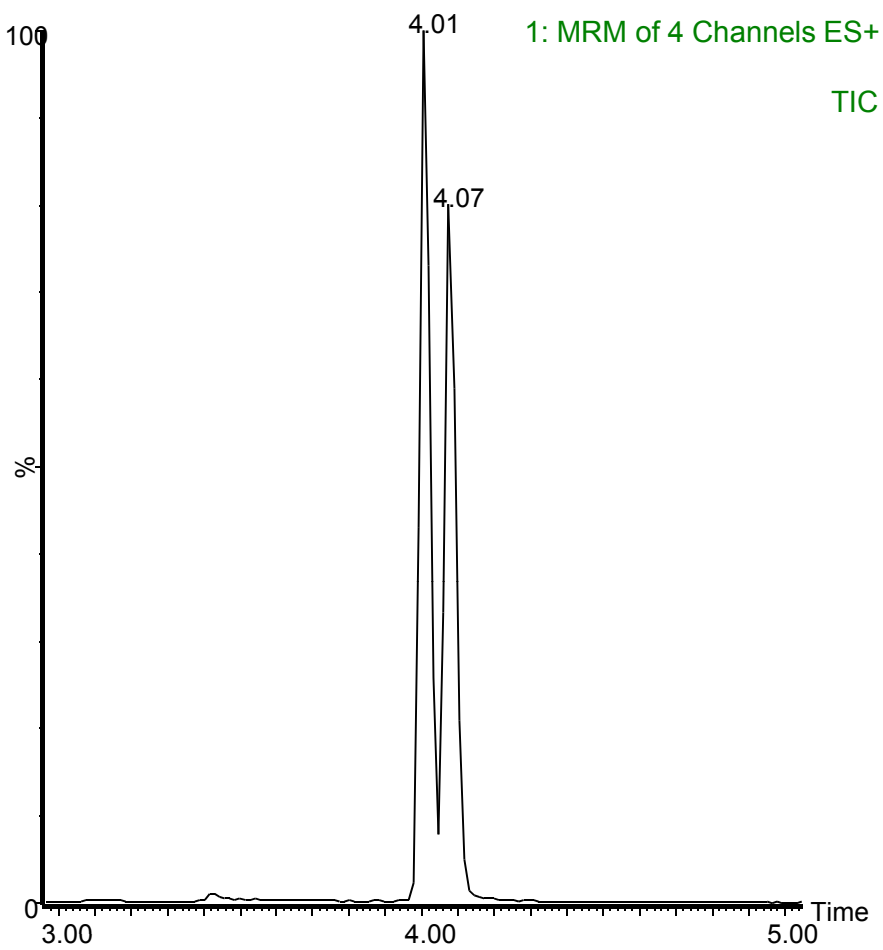


Heart cut determination
ESI source

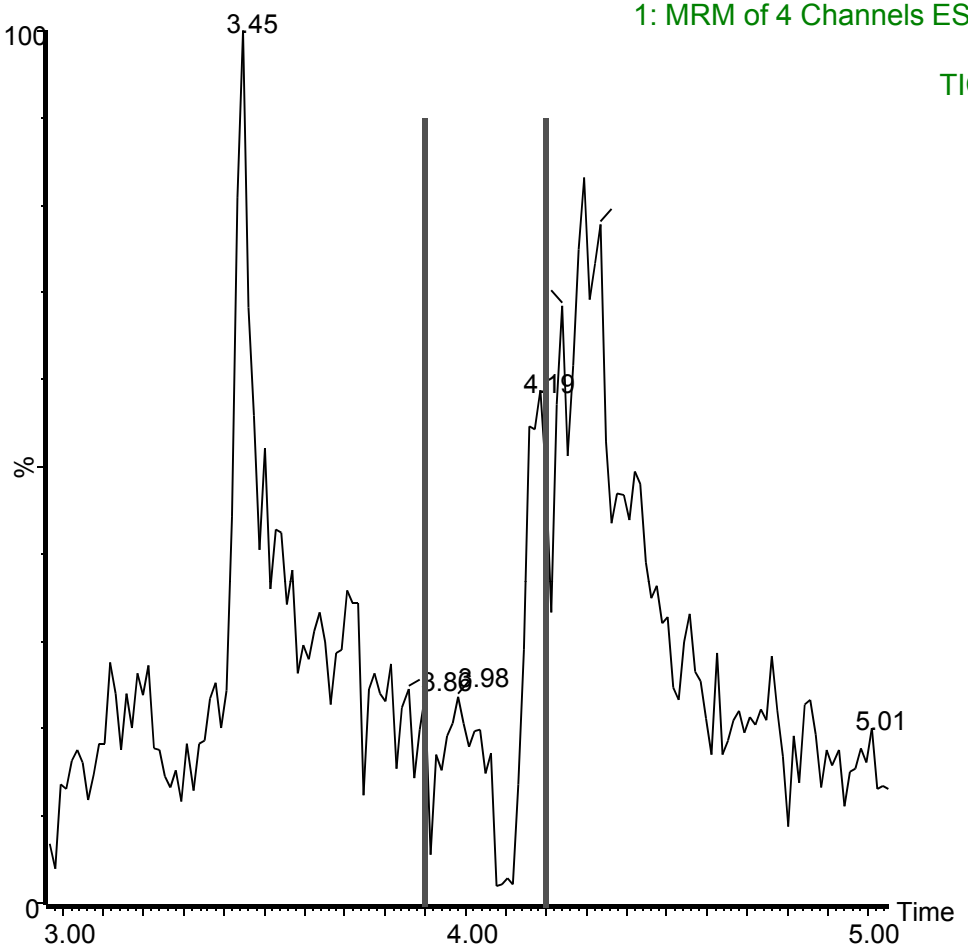


Trap2: gradient 50µl/min
Aux pump A: H_2O flowrate 150µl/min

Preliminary work: optimizing heart-cut window



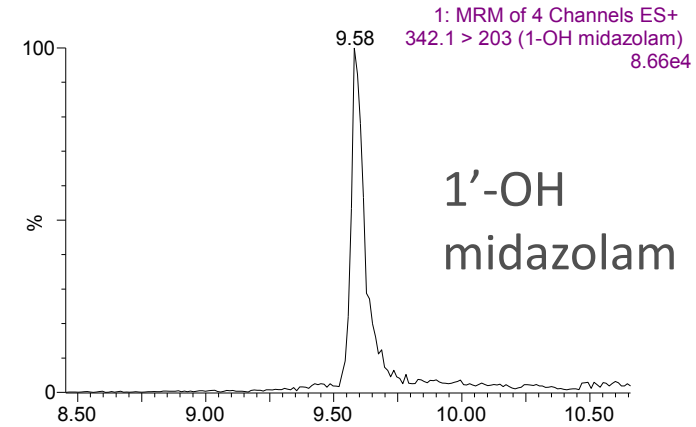
NO HC

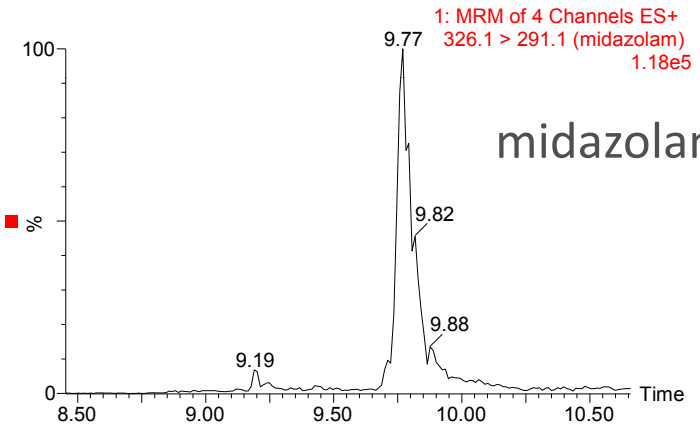


HC 3.9-4.2 min

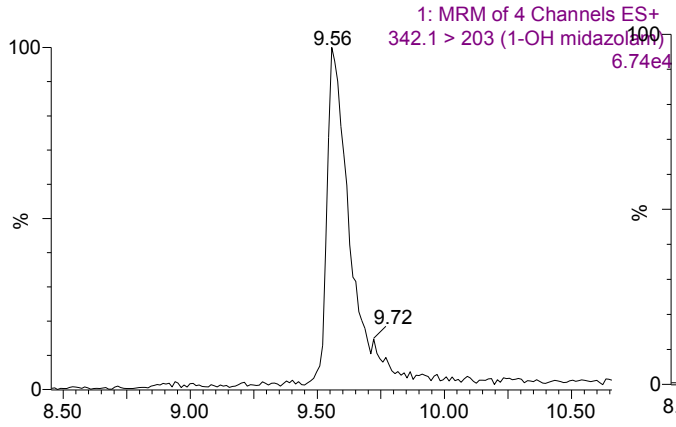
Preliminary work: 2D trap selection 0.5x5mm 3µ

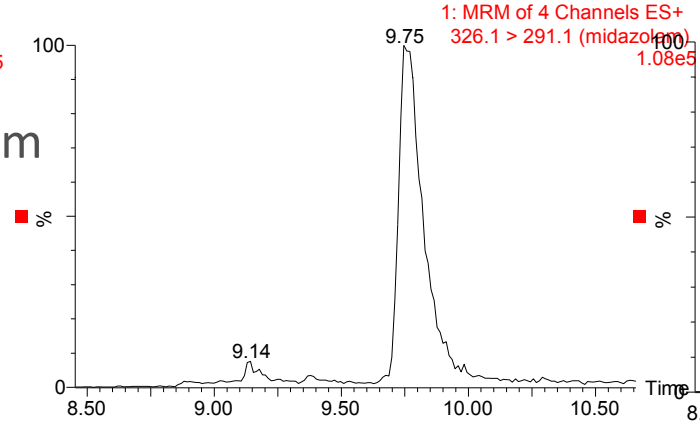
YMC Triart C18



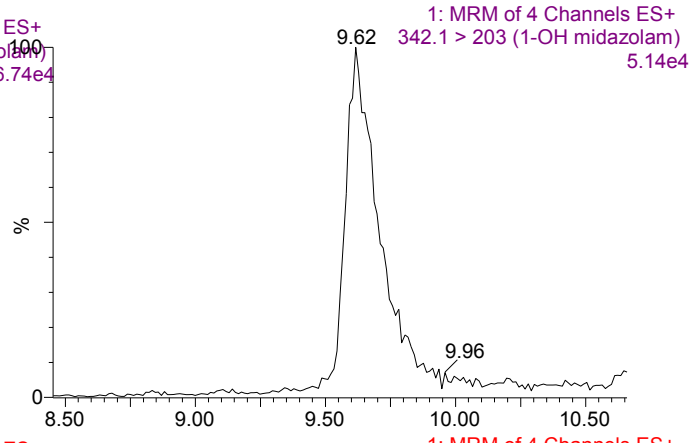


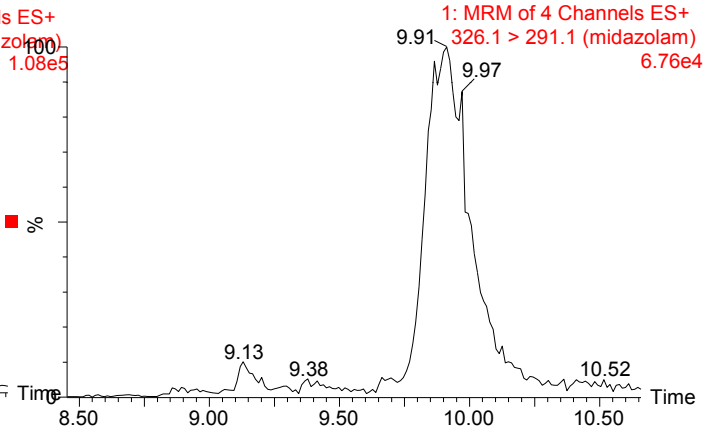
YMCTriart C8



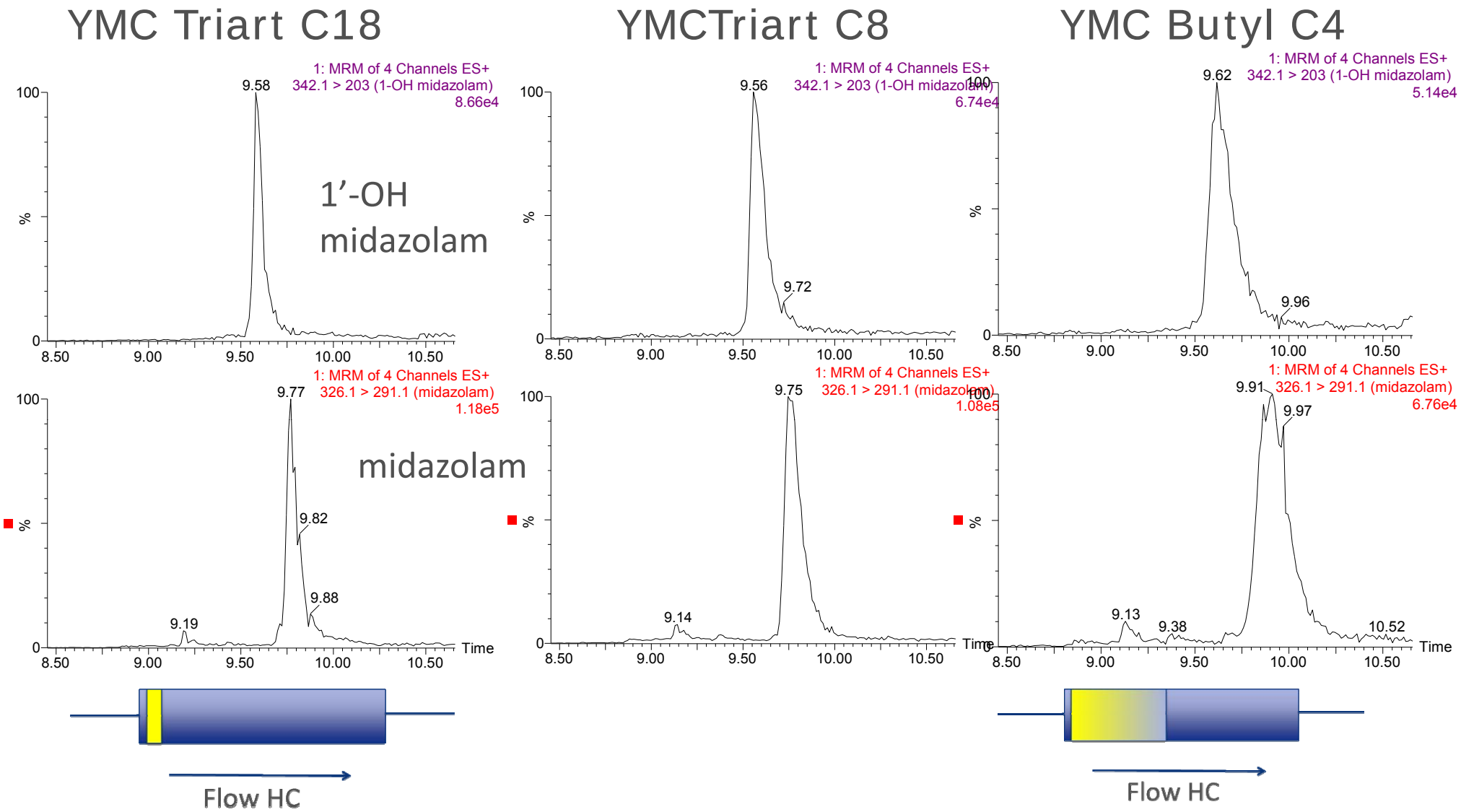


YMC Butyl C4





Preliminary work: 2D trap selection 0.5x5mm 3µ



Outline

- 2D LC concept and our aim
- Our 2D micro-system and how it works
- Preliminary work: direct column injections, heart cut determination,…
- **Practical considerations during optimization**
- Results
- Conclusion

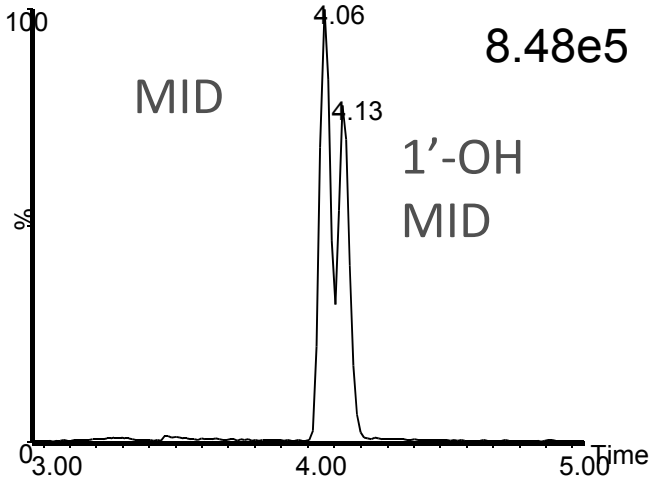
Practical considerations during optimization

- Back pressure
  - Modules have different max pressures
    - AUX BSM 10000 psi
    - M-class BSM 15000 psi
    - Nano BSM 10000 psi (depending on the tile used in the ionKey)
    - Limitation in flow rates, compatible tubing dimensions, dilution before trapping
  - Selection of second trap column : the right dimension

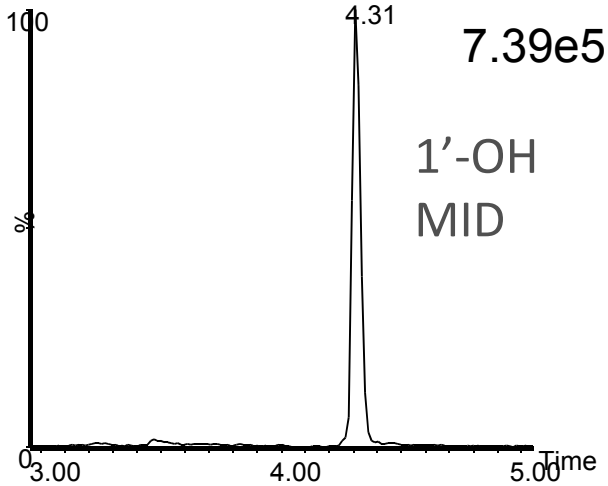
Practical considerations during optimization

- Back pressure
  - Modules have different max pressures
    - AUX BSM 10000 psi
    - M-class BSM 15000 psi
    - Nano BSM 10000 psi (depending on the tile used in the ionKey)
    - Limitation in flow rates, compatible tubing dimensions, dilution before trapping

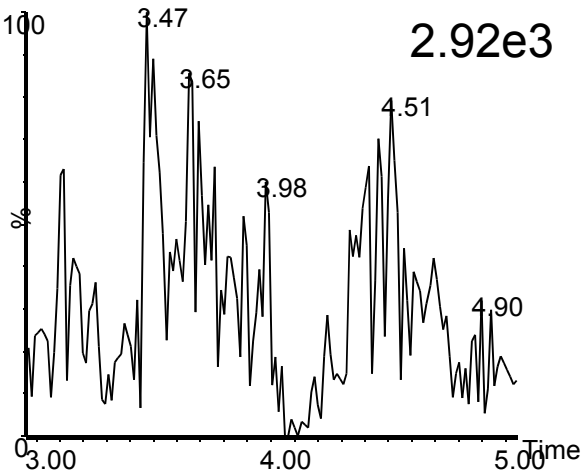
  - Selection of second trap column : the right dimension



NO HC



HC 3.9-4.2



HC 3.9-4.35

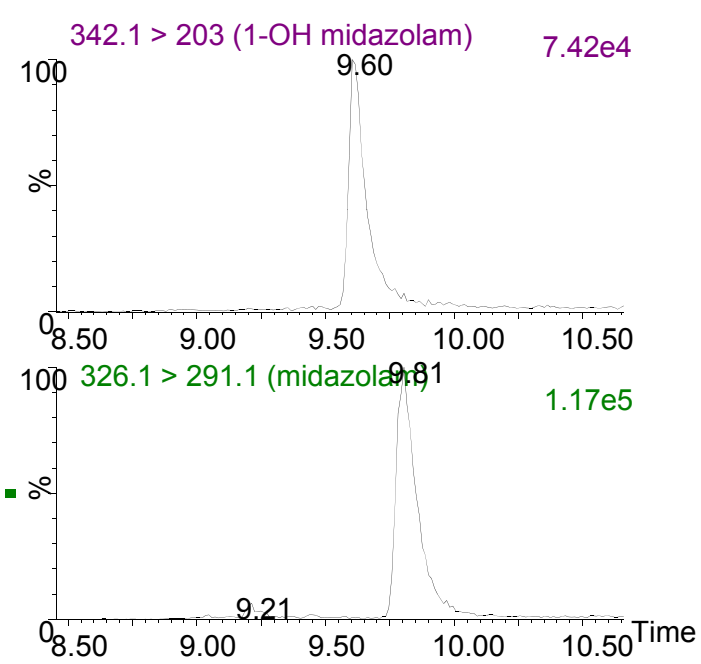
Practical considerations during optimization

- Back pressure
  - Modules have different max pressures
  - Selection of second trap column : the right dimension
- Carry over
  - fg/ml concentration range
  - Injection loop: solvent B H_20/ACN/IPA
  - Cycle sample loop 10x

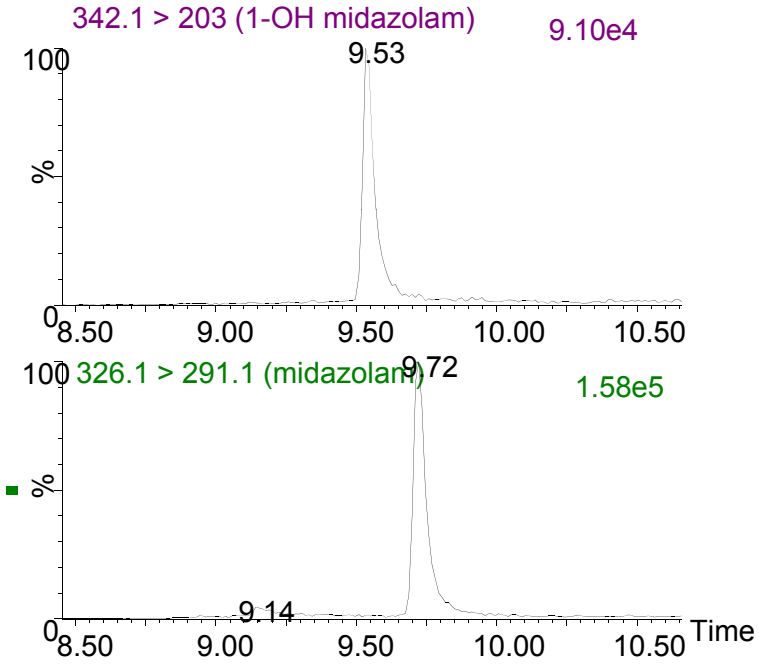
Practical considerations during optimization

- Back pressure
- Carry over
- Peak tailing

Dilute with H_2O



Dilute with NH_4oAc 0.1M

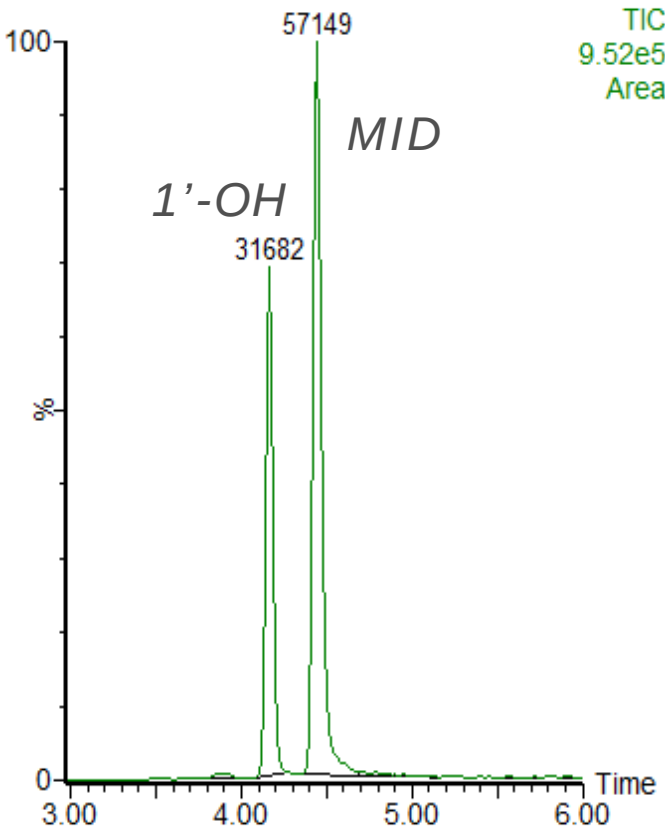


Outline

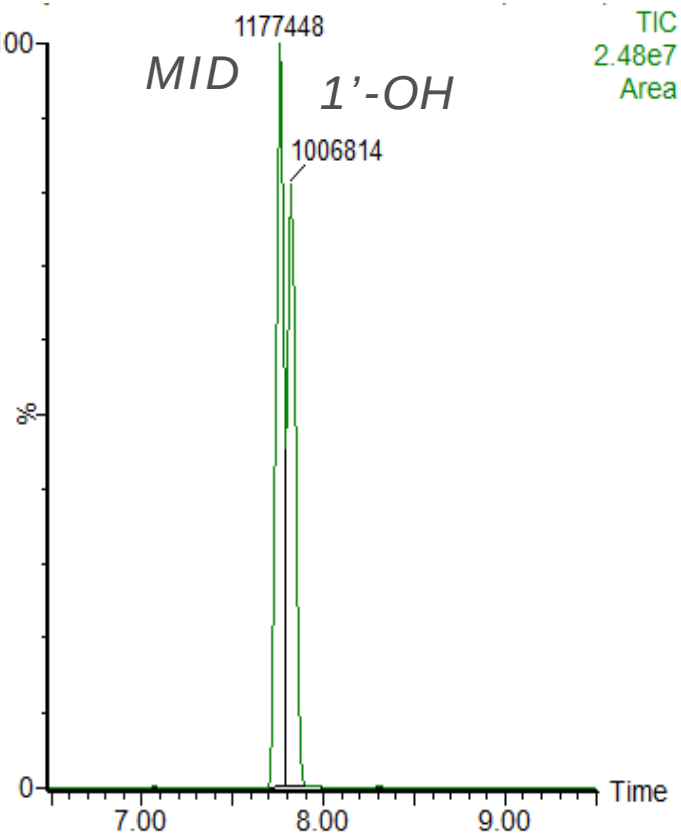
- 2D LC concept and our aim
- Our 2D micro-system and how it works
- Preliminary work: direct column injections, heart cut determination,…
- Practical considerations during optimization
- **Results**
- Conclusion

Results: gain of sensitivity

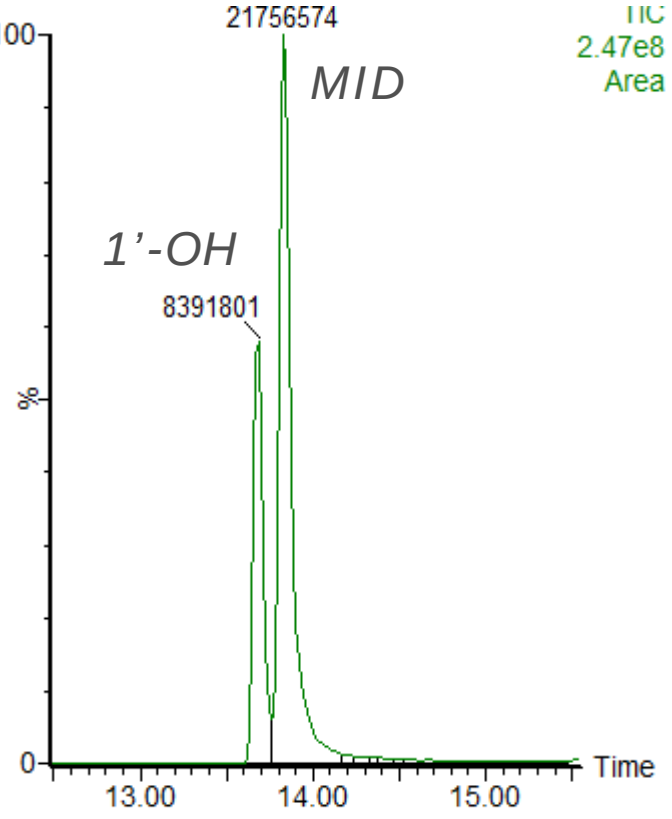
0.2 µL injection
2 µL/min ionKeyMS



50 µL injection
50 µL/min
trap+ 1x50 mm CSH phenyl



50 µL injection
2 µL/min
2D-LC ionKeyMS

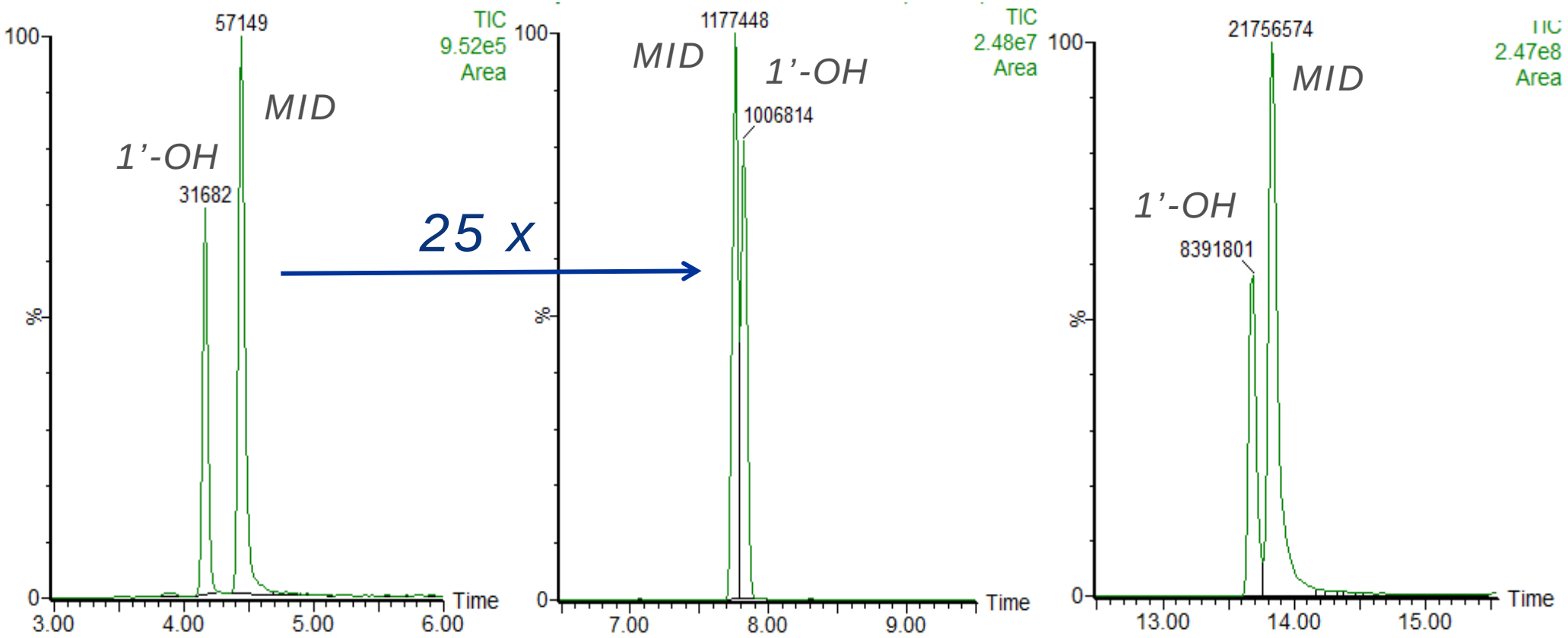


Results: gain of sensitivity

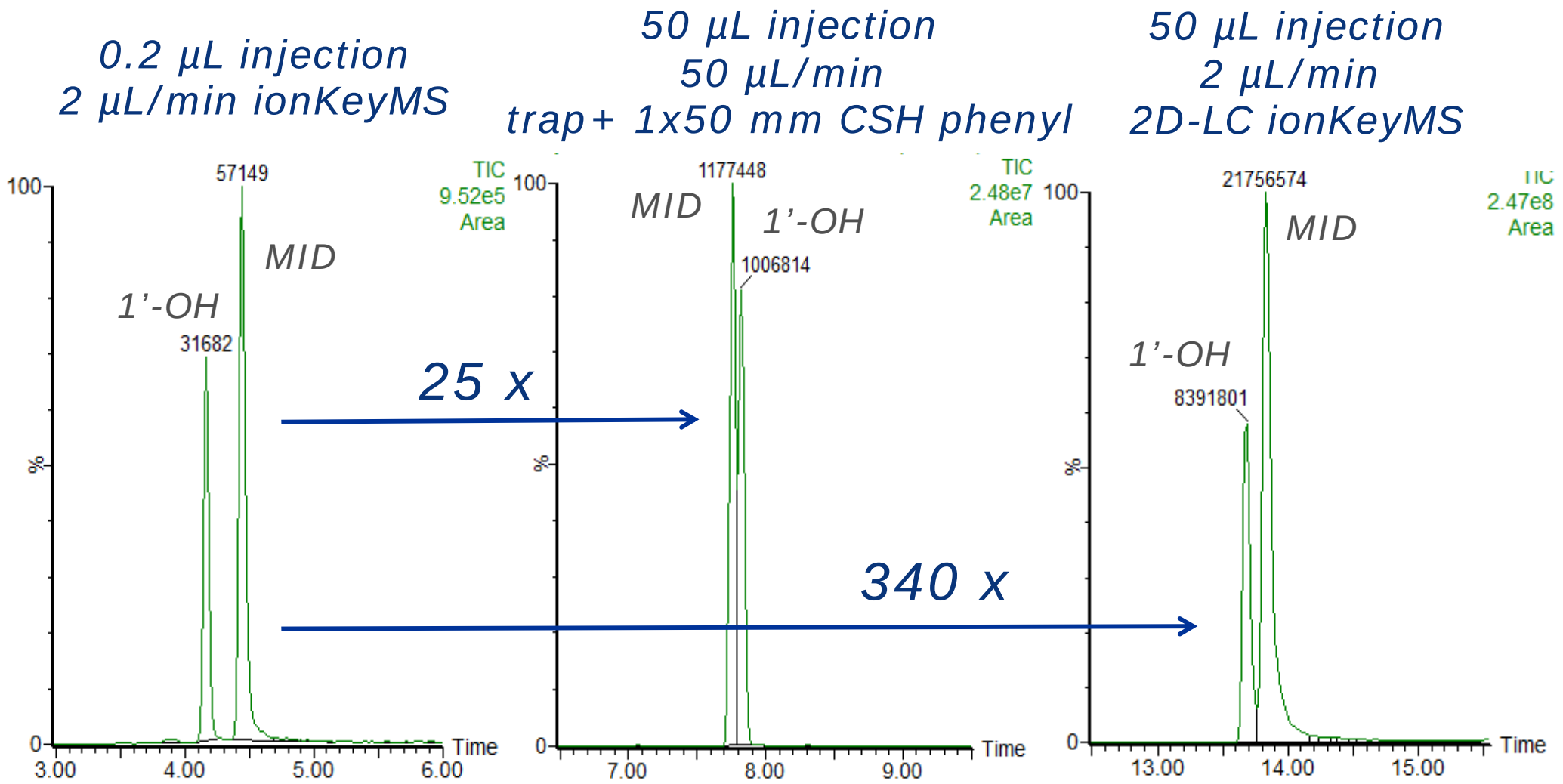
0.2 µL injection
2 µL/min ionKeyMS

50 µL injection
50 µL/min
trap+ 1x50 mm CSH phenyl

50 µL injection
2 µL/min
2D-LC ionKeyMS



Results: gain of sensitivity



Results: gain of sensitivity

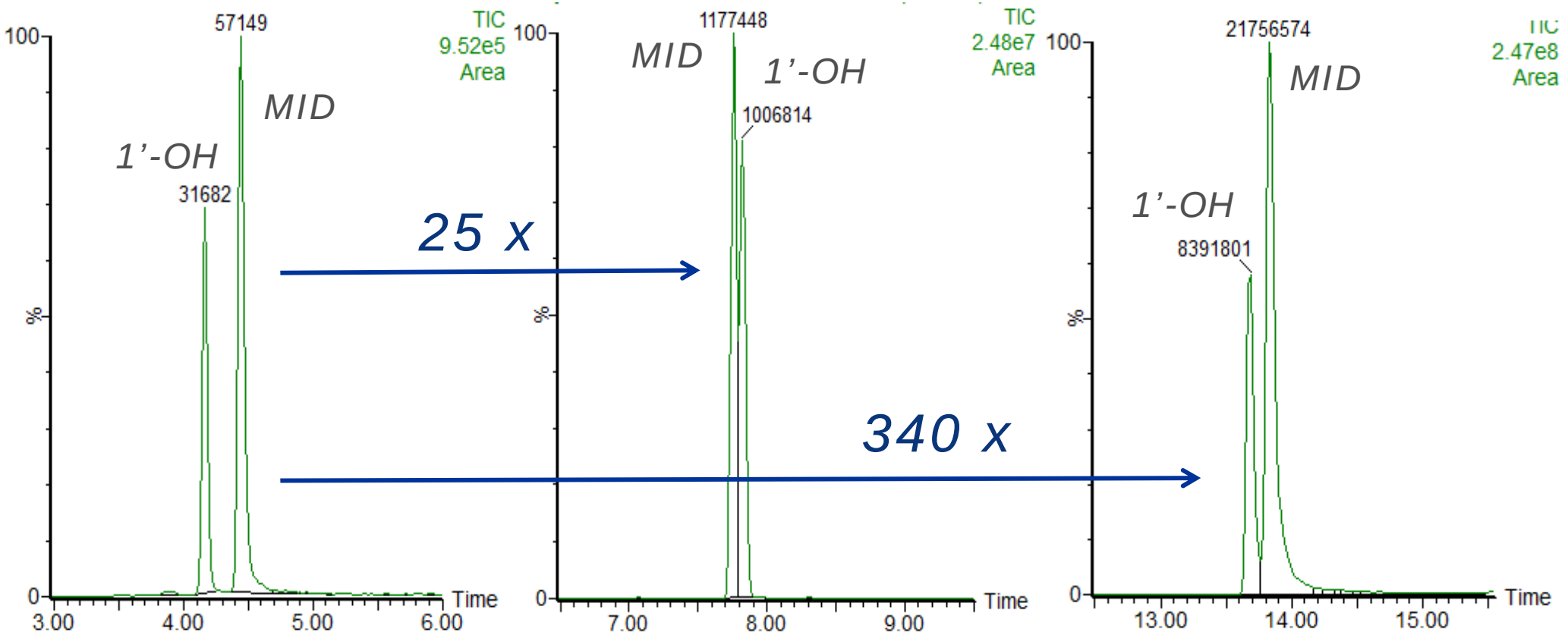
Equivalent of ± 40 µL injected on a 2.1 x 50 mm column

0.2 µL injection
2 µL/min ionKeyMS

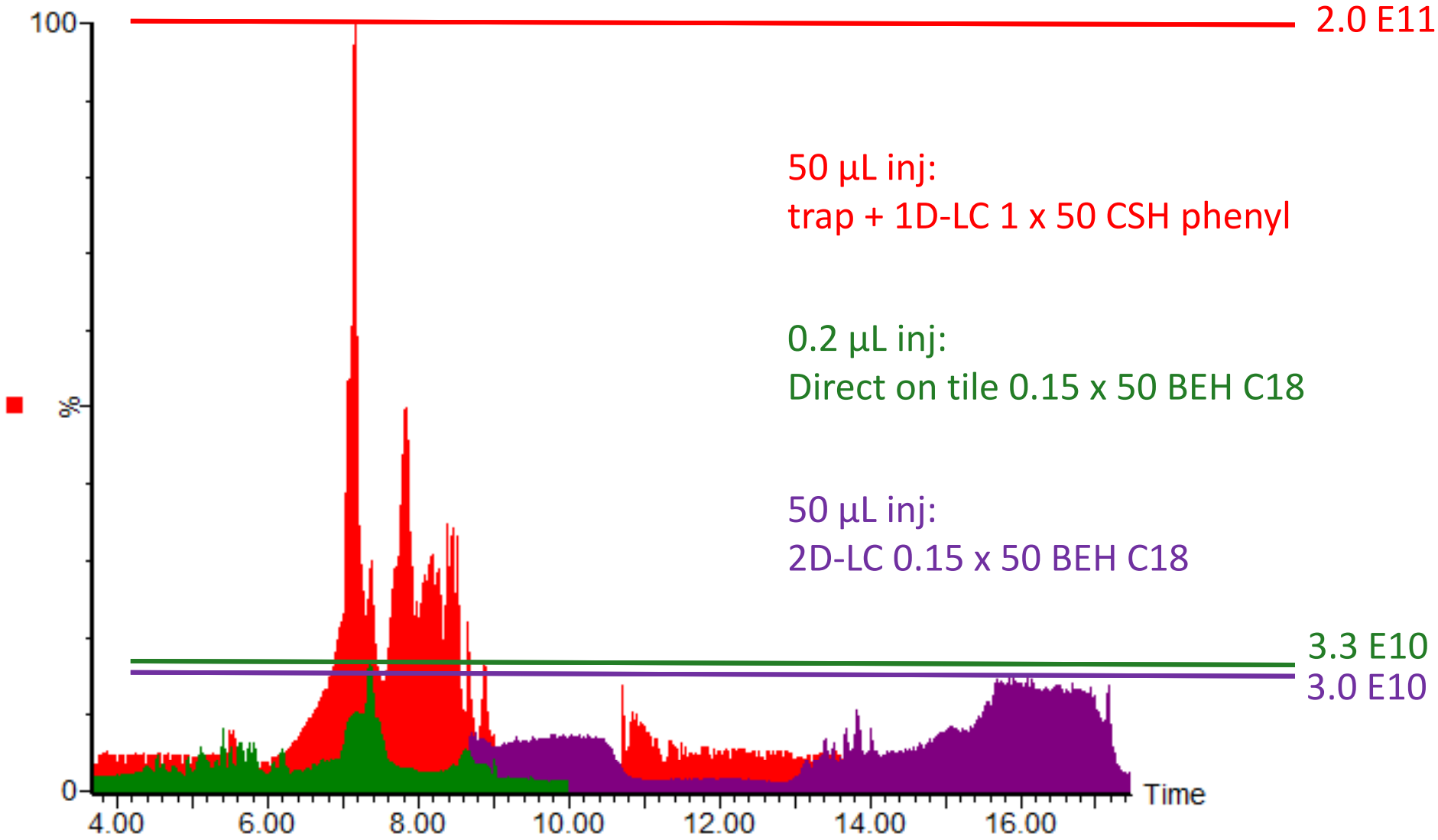
50 µL injection
50 µL/min
trap+ 1x50 mm CSH phenyl

Equivalent of ± 10 mL injected on a 2.1 x 50 mm column

50 µL injection
2 µL/min
2D-LC ionKeyMS



Results: TIC (full MS) background comparison



Results: analysis human plasma

Plasma precipitation

curve and QC's prepared in human EDTA plasma

100µl plasma + 50µl IS DMSO + 300µl Acetonitrile

» 50µl SN injected » 11µl plasma on column

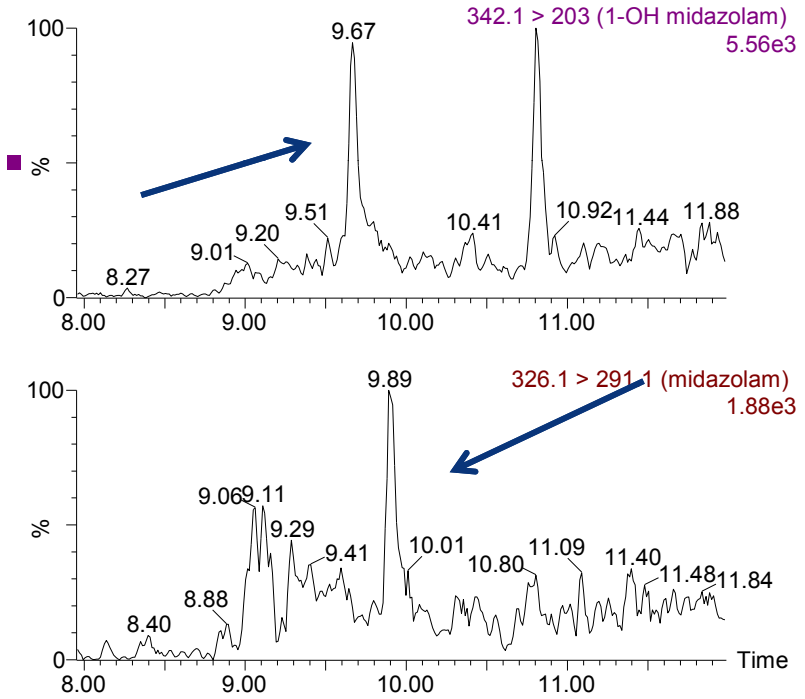
Dynamic range:

1'-OH Midazolam:

0.100pg/ml – 100pg/ml

Midazolam:

0.100pg/ml – 100pg/ml



Results: analysis human plasma

Midazolam	std 1	std 2	std 3	std 4	std 5	std 6	std 7	std 8	std 9	std 10
conc pg/ml	0.100	0.250	0.500	1.00	2.50	5.00	10.0	25.0	50.0	100
mean	0.0890	0.268	0.526	1.09	2.48	4.97	9.19	25.9	47.7	103
stdev	0.004	0.039	0.013	0.083	0.143	0.063	0.234	3.03	0.353	1.59
% CV	4%	15%	2%	8%	6%	1%	3%	12%	1%	2%
	conc pg/ml		mean	stdev	% CV					
QC1	0.1		0.133	0.024	18%					
QC2	0.5		0.559	0.057	10%					
QC3	10		10.6	0.172	2%					
QC4	50		48.3	1.18	2%					

Results: analysis human plasma

1'-OH Midazolam	std 1	std 2	std 3	std 4	std 5	std 6	std 7	std 8	std 9	std 10
conc pg/ml	0.100	0.250	0.500	1.00	2.50	5.00	10.0	25.0	50.0	100
mean	0.1110	0.263	0.468	0.921	2.37	4.86	9.88	26.6	49.1	106
stdev	0.0014	0.042	0.014	0.033	0.023	0.06	0.16	0.947	2.29	4.48
% CV	1%	16%	3%	4%	1%	1%	2%	4%	5%	4%
	conc pg/ml		mean	stdev	% CV					
QC1	0.1		0.105	0.021	20%					
QC2	0.5		0.491	0.027	5%					
QC3	10		10.3	0.43	4%					
QC4	50		52.7	1.33	3%					

Conclusion

- Complete 2D micro-UHPLC system with heart cut
- Gain in sensitivity → fg/ml concentration range
- Reduction of background/ionsuppression for large volume injection
- Challenges: backpressure, carry over and peak tailing

Future work:
» Apply this generic approach on other small molecules and peptides
» Publish our work

Acknowledgements

Janssen R&D

Pharmacokinetics, Dynamics & Metabolism, Belgium

Ronald de Vries

Filip Cuyckens

Lieve Dillen

Rob Vreeken

Willy Cools

Waters

Isabelle Francois

Ben van de Perre

Steve Preece

Thank you

lvereyk1@its.jnj.com

November 19, 2015

janssen


janssen