February 03, 2025

Development of a heart-cut SFC-SFC setup

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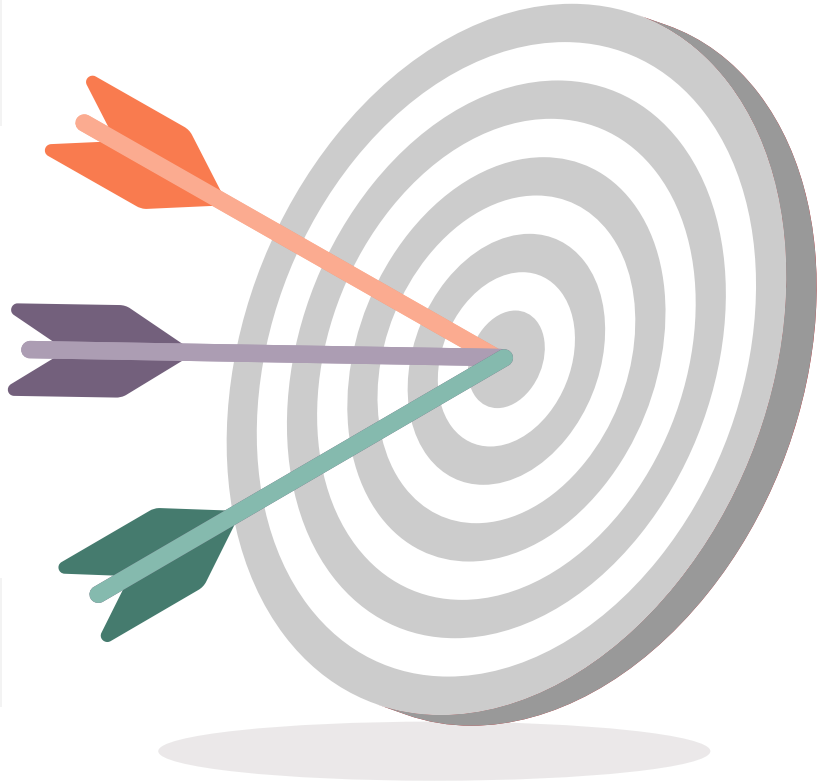
Objectives

Introduction

Develop a 2D system SFC-SFC in multiple heart-cutting

Understand the effects linked to the coupling of the 2 dimensions

Determine the diastereoisomeric excesses of different parts of bitter orange



Citrus aurantium L.

Matrix - interest

Introduction

01

Citrus aurantium, sour orange, bitter orange

02

Rich in bioactive molecules, especially flavonoids

03

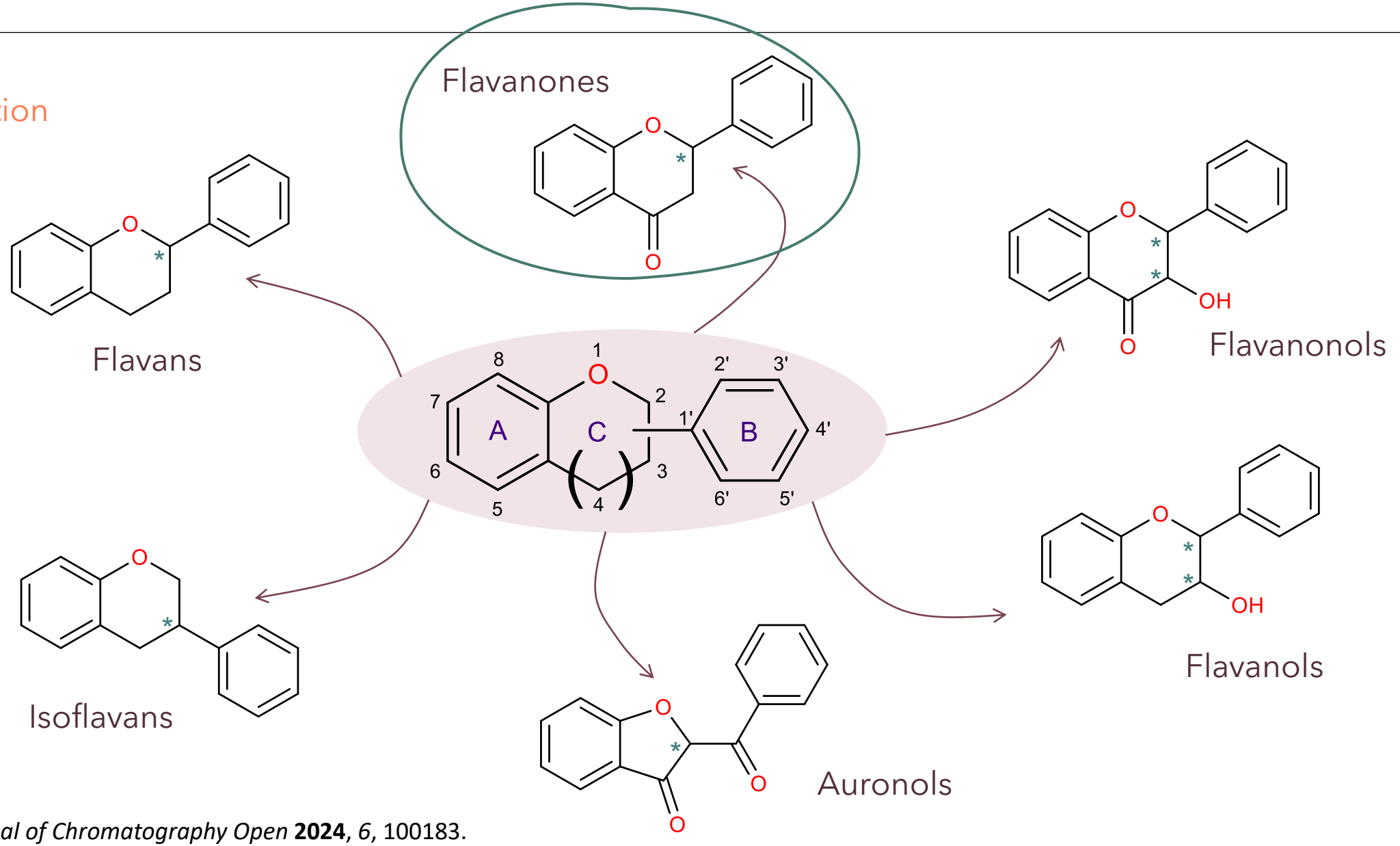
Cosmetic and pharmaceutical interests

04

Anticancer, cardiovascular, anti-inflammatory…

Chiral flavonoids

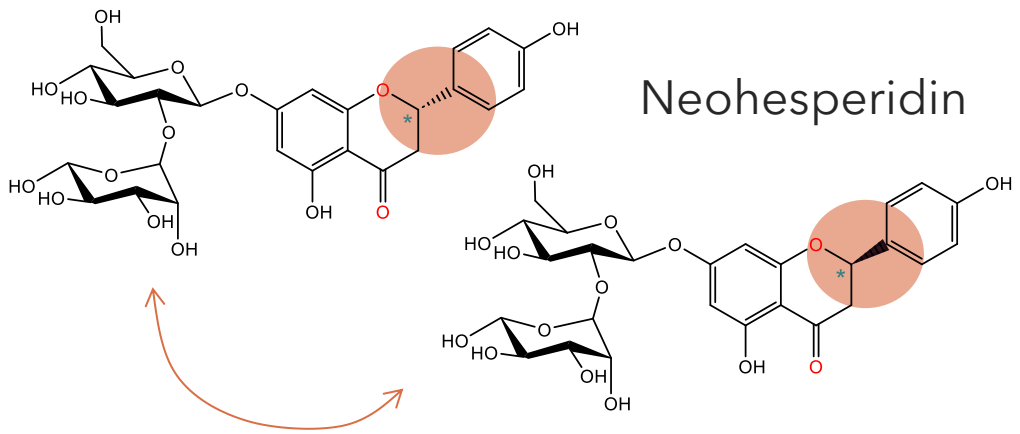
Introduction

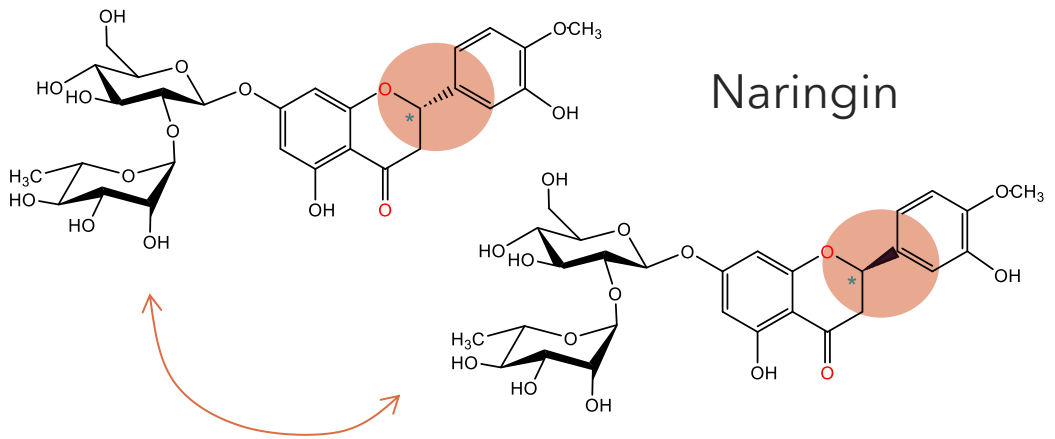


Réset, L. *et al Journal of Chromatography Open* **2024**, *6*, 100183.

Matrix – interest of bitter orange

Introduction

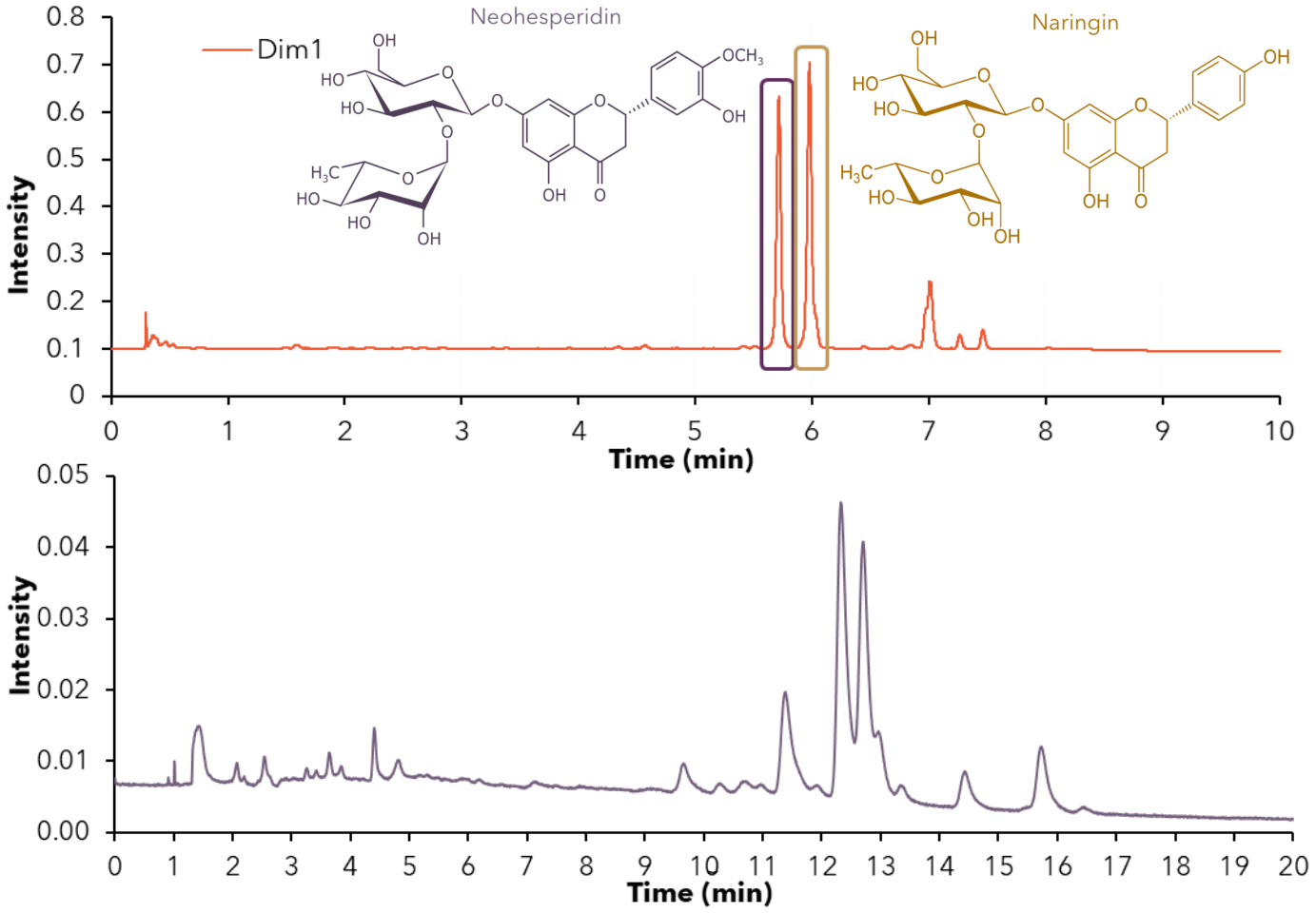




Different properties

It is necessary to succeed in separating the different diastereoisomers because they have different properties. With the aim of valorizing the plants, particularly for pharmaceutical applications.

Interest of 2D system



dim1

Achiral

Column Torus DEA

MeOH + 0.1% MSA

Separation of flavonoids

dim2

Chiral

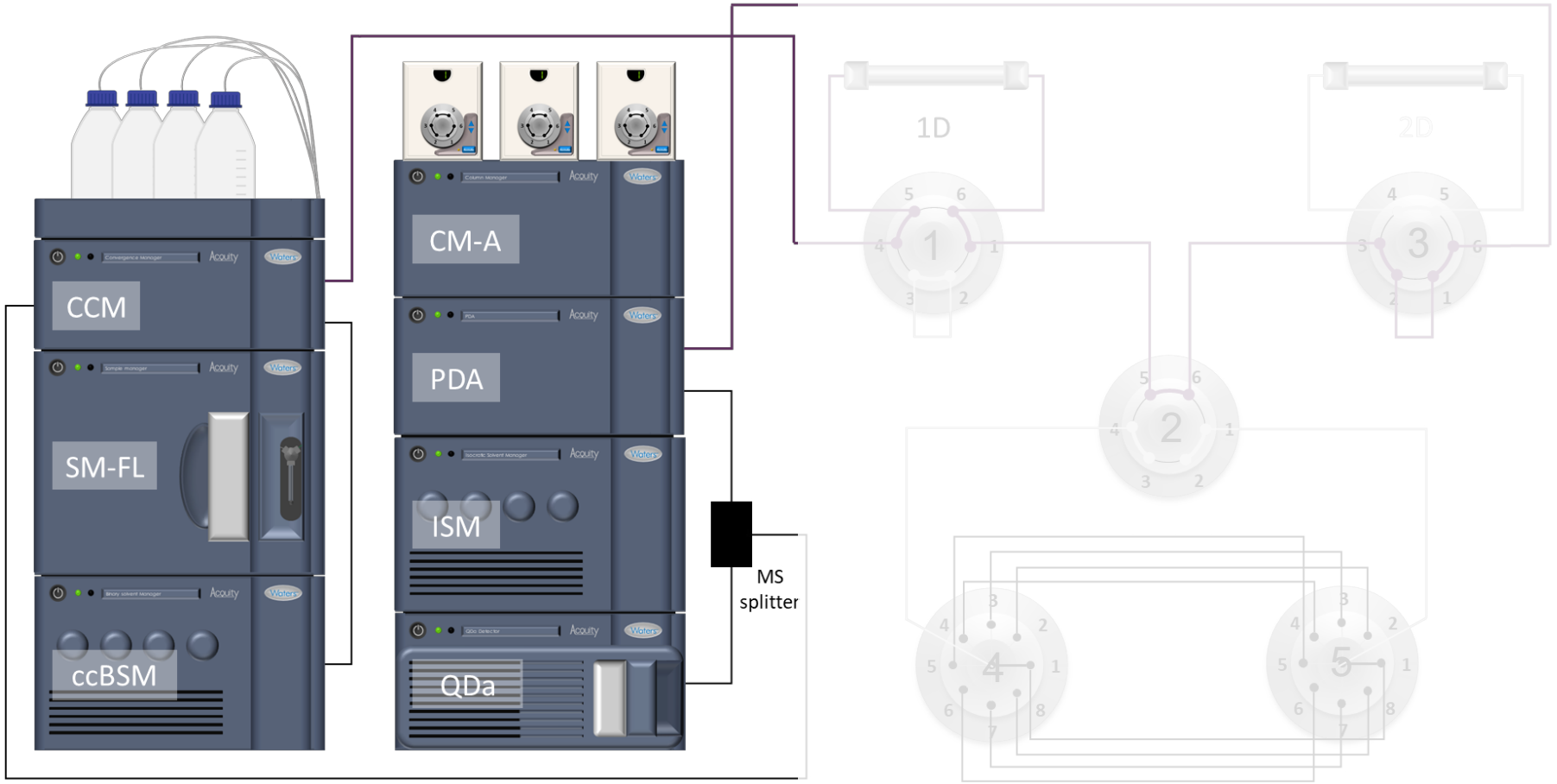
Column Chiralpak IB

MeOH + 0.1% MSA

Separation of diastereoisomers

2D system – dim1

2D system

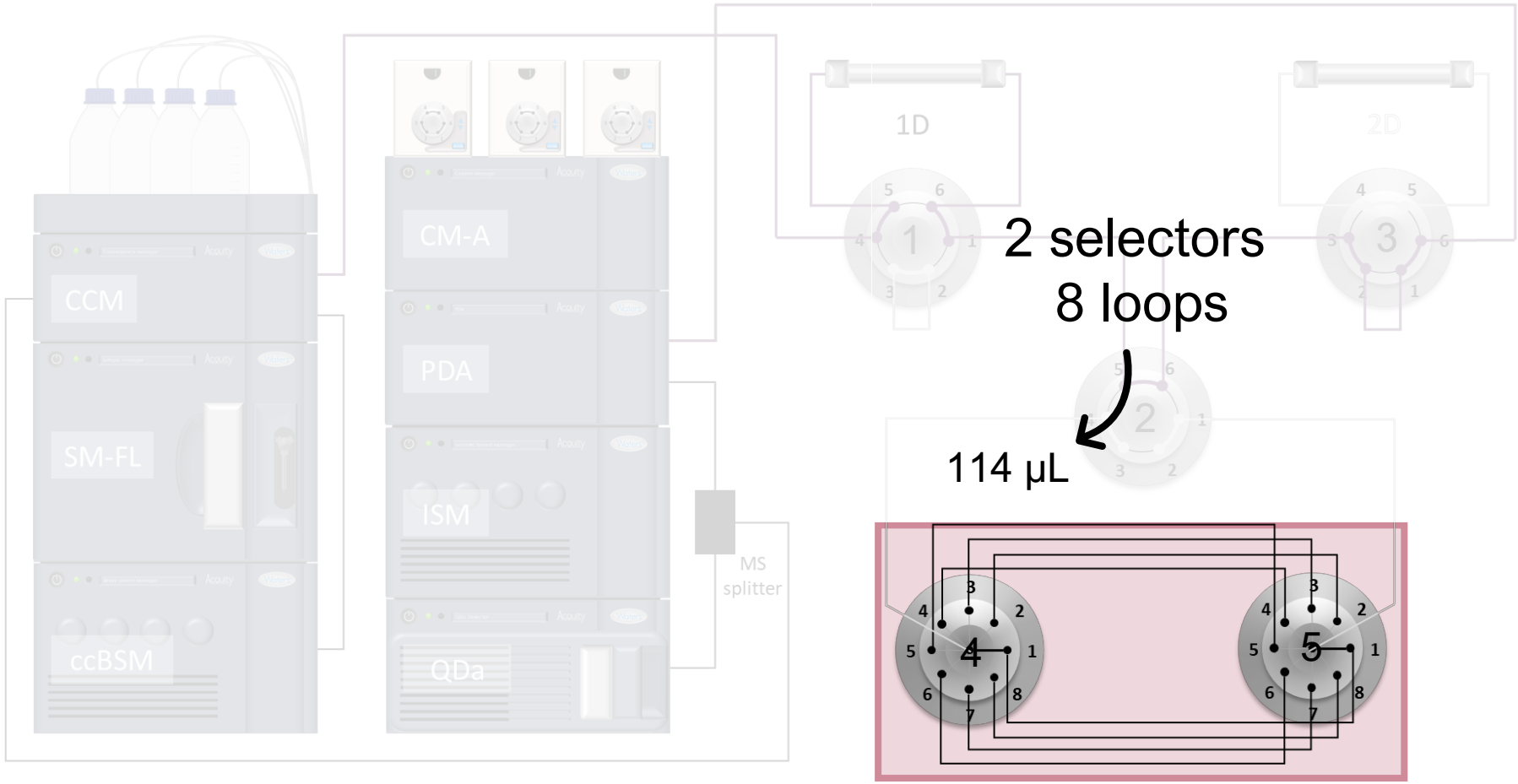


Waters™
ACQUITY UPC²

Use of a conventional SFC system

2D system – dim1

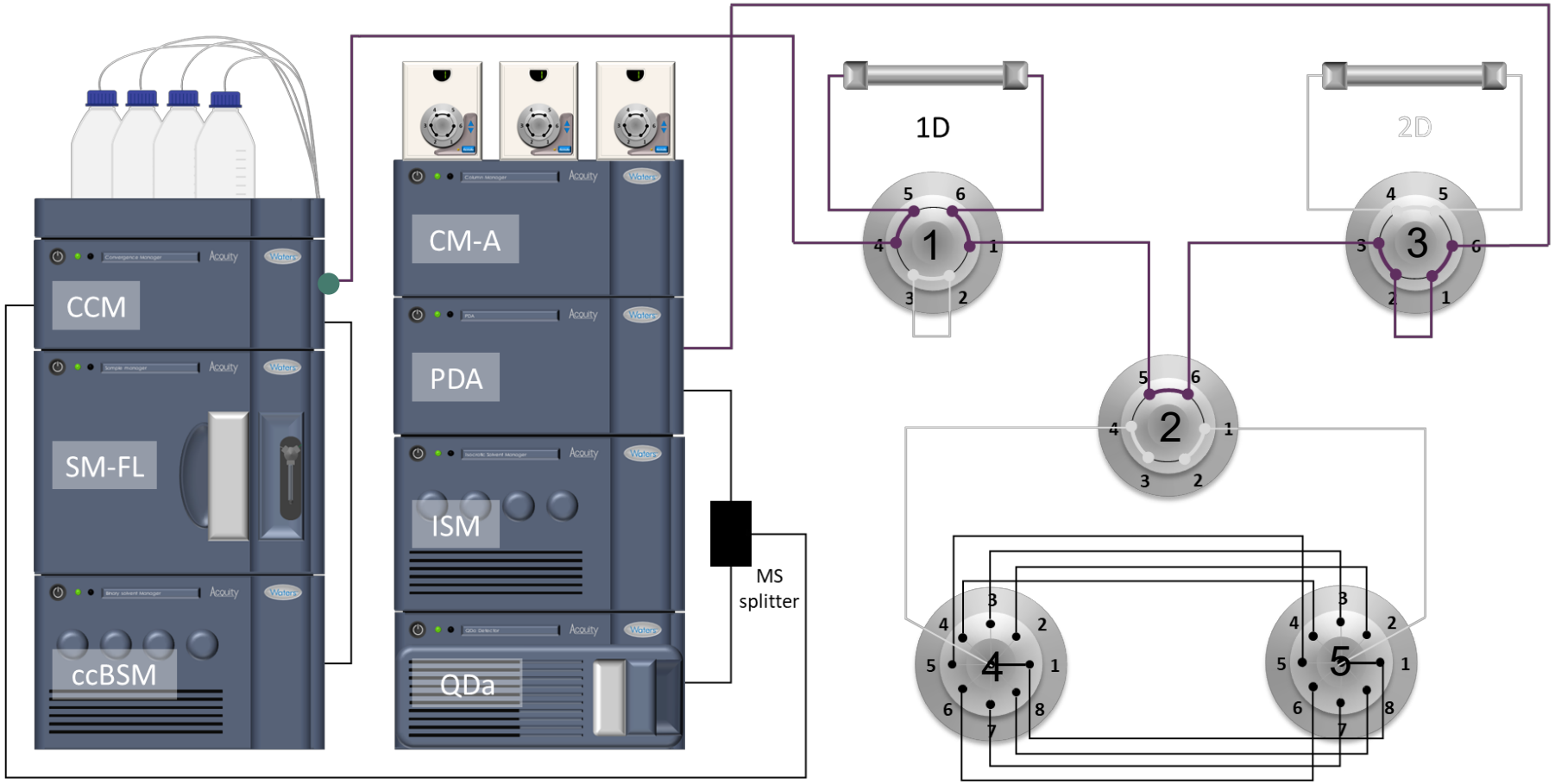
2D system



Use of the selectors initially present with 114 µL loops

2D system – dim1

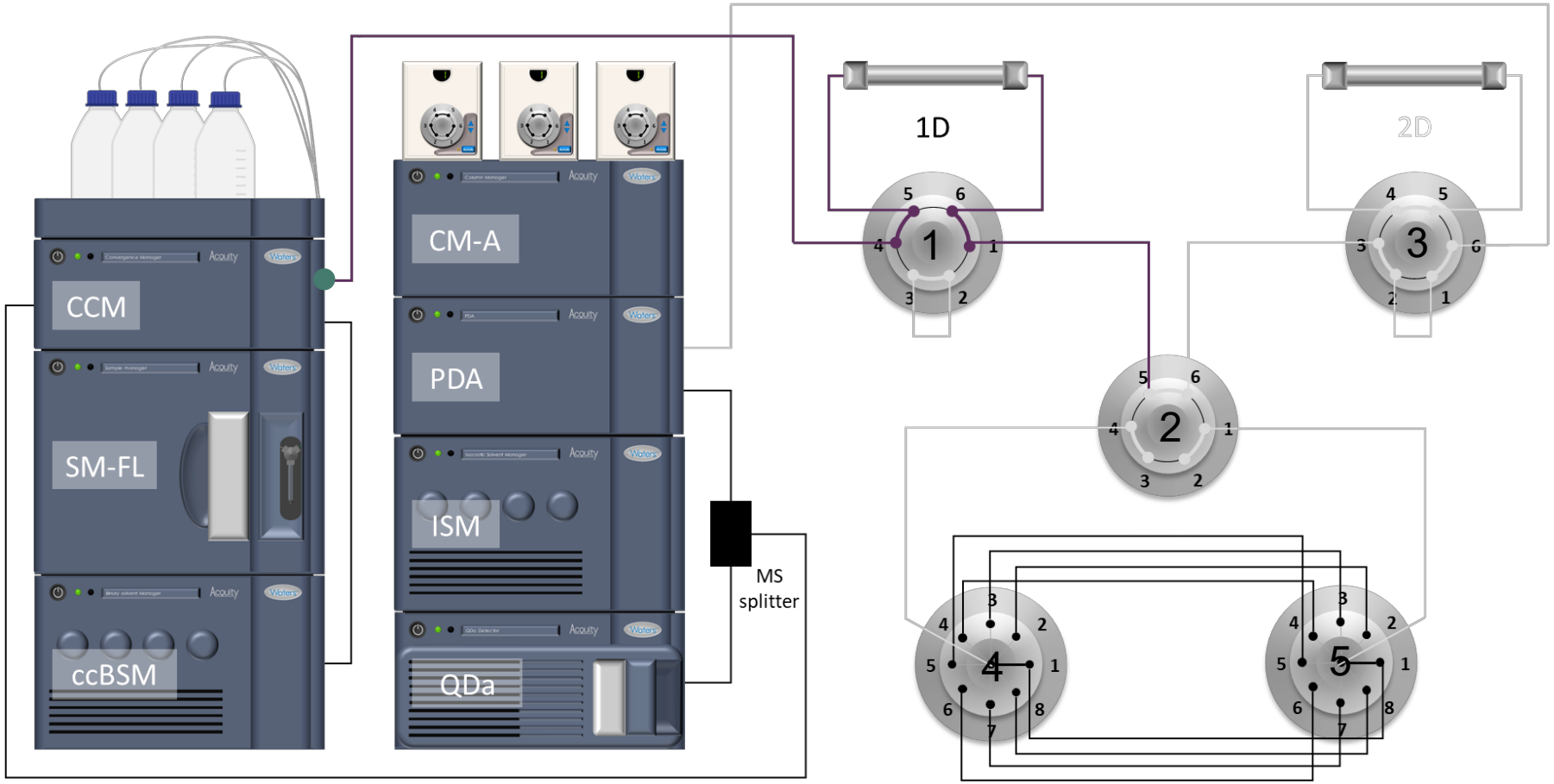
2D system



Waters UPC² 1D system + 3 additional 6-port 2-position valves

2D system – dim1 load loop

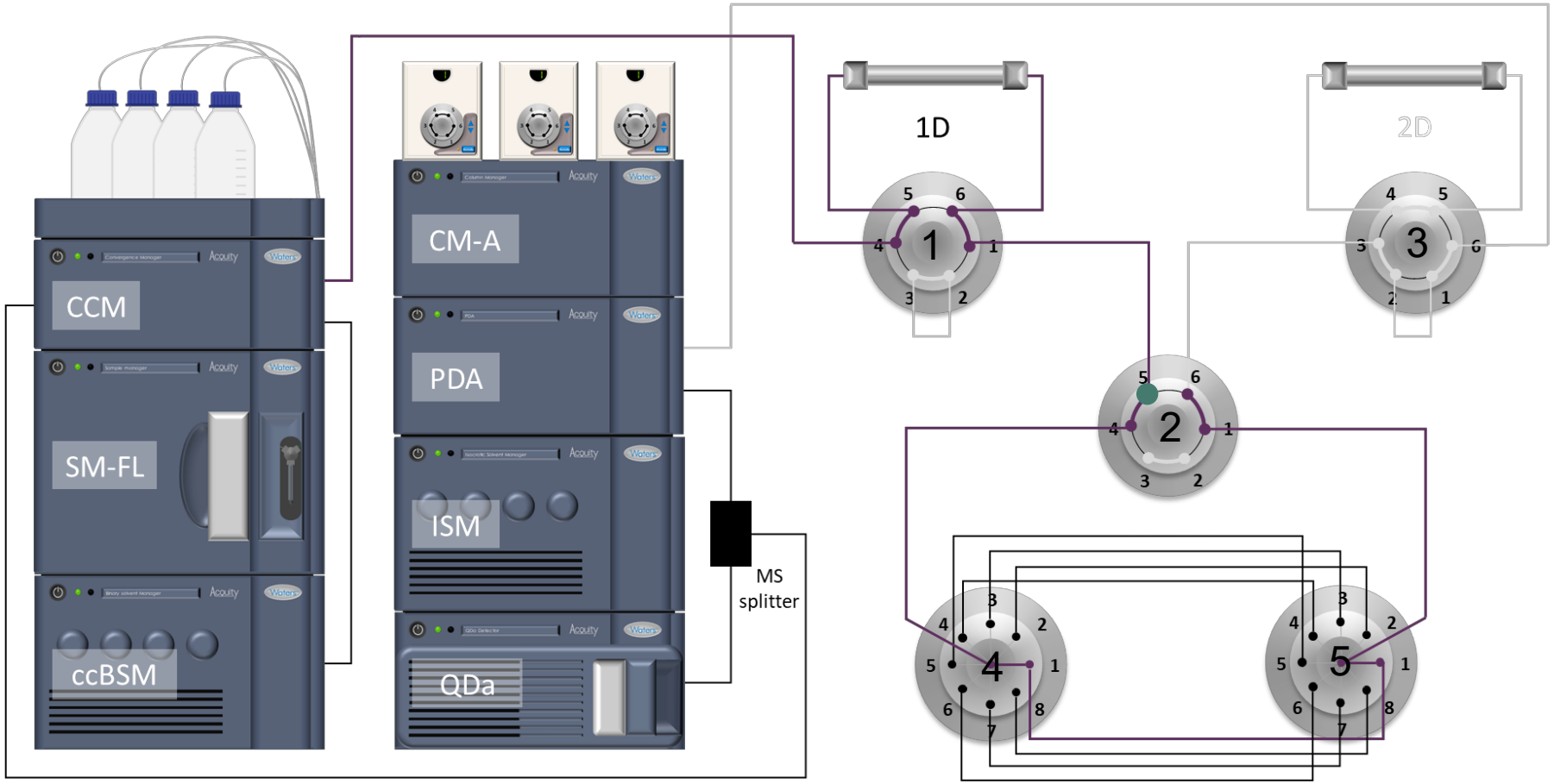
2D system



Analysis in the first dimension and loading loops to store peaks of interest

2D system – dim1 load loop

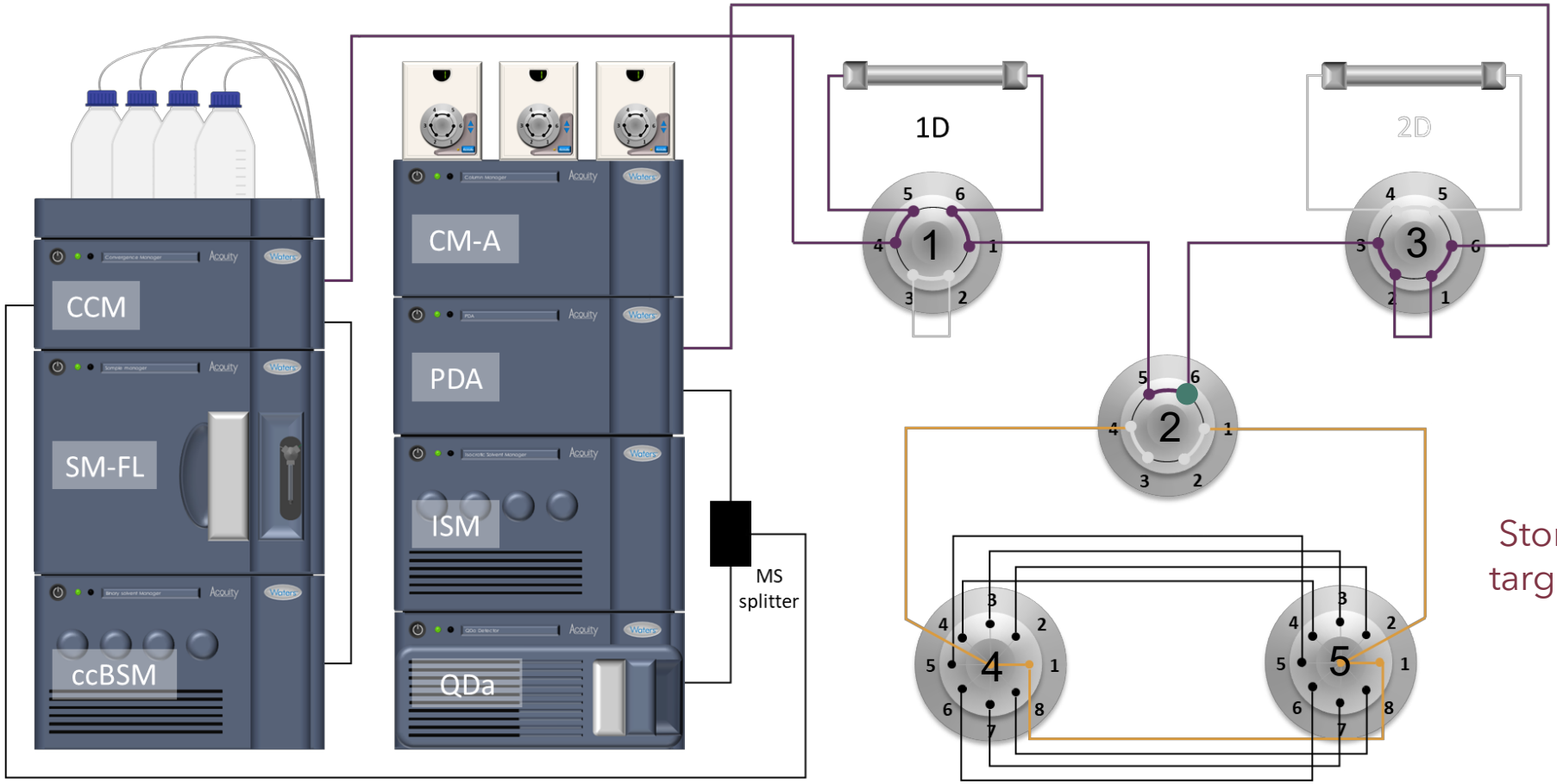
2D system



Analysis in the first dimension and loading loops to store peaks of interest

2D system – dim1 load loop

2D system

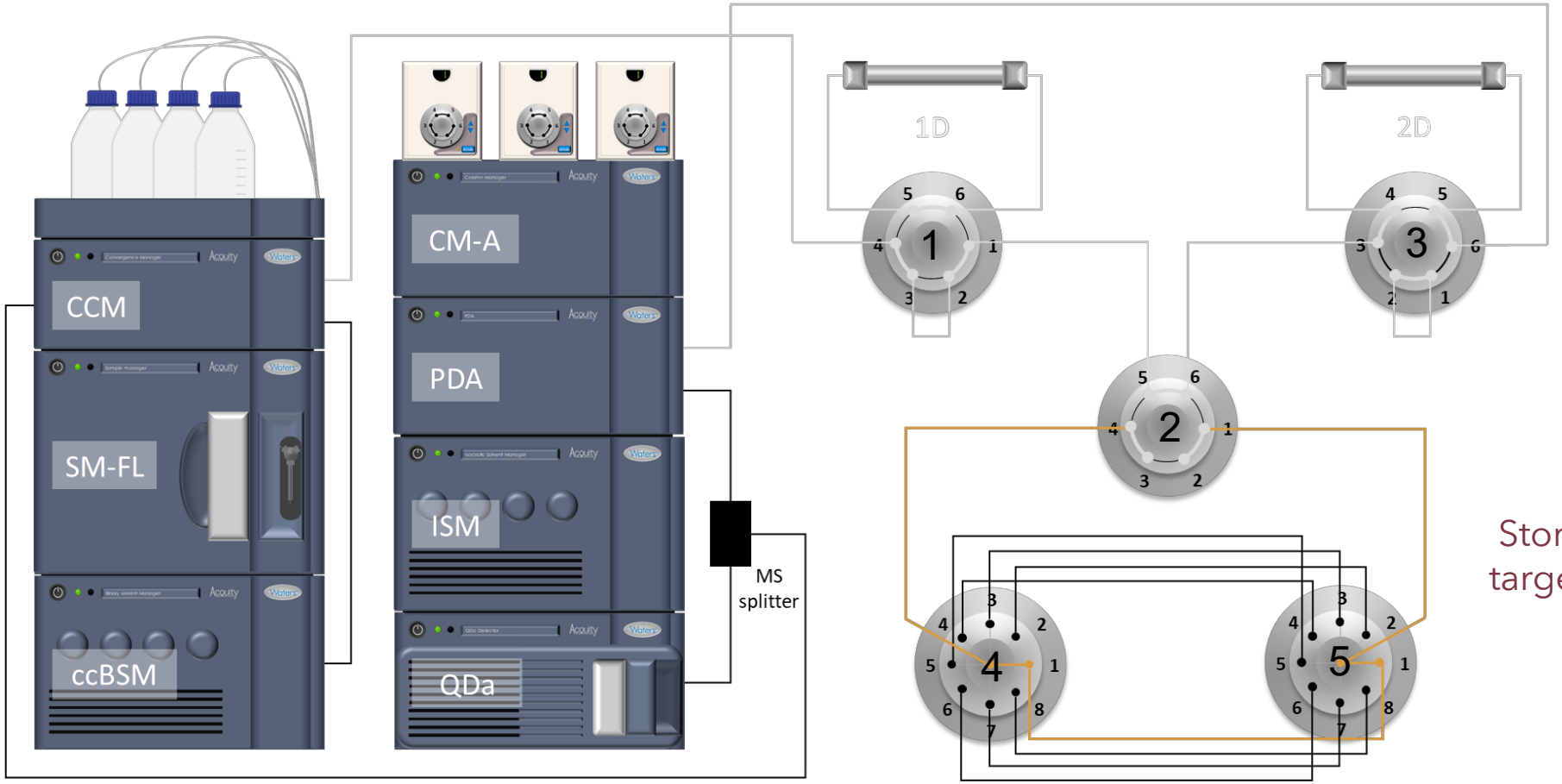


Storage of target peak

The fraction remains stored in the loop and the analysis continues

2D system – unload loop in dim2

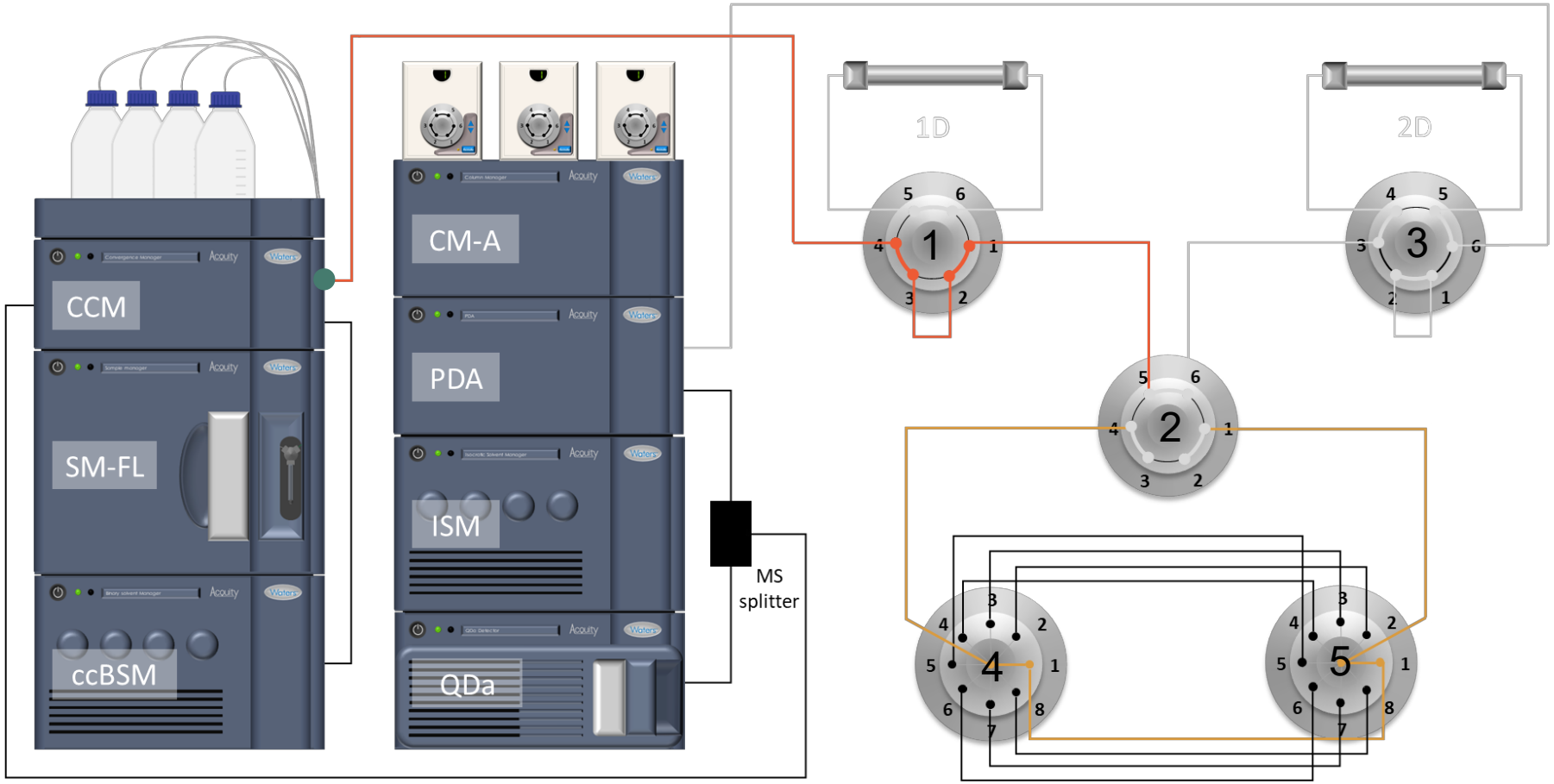
2D system



Storage of target peak

2D system – unload loop in dim2

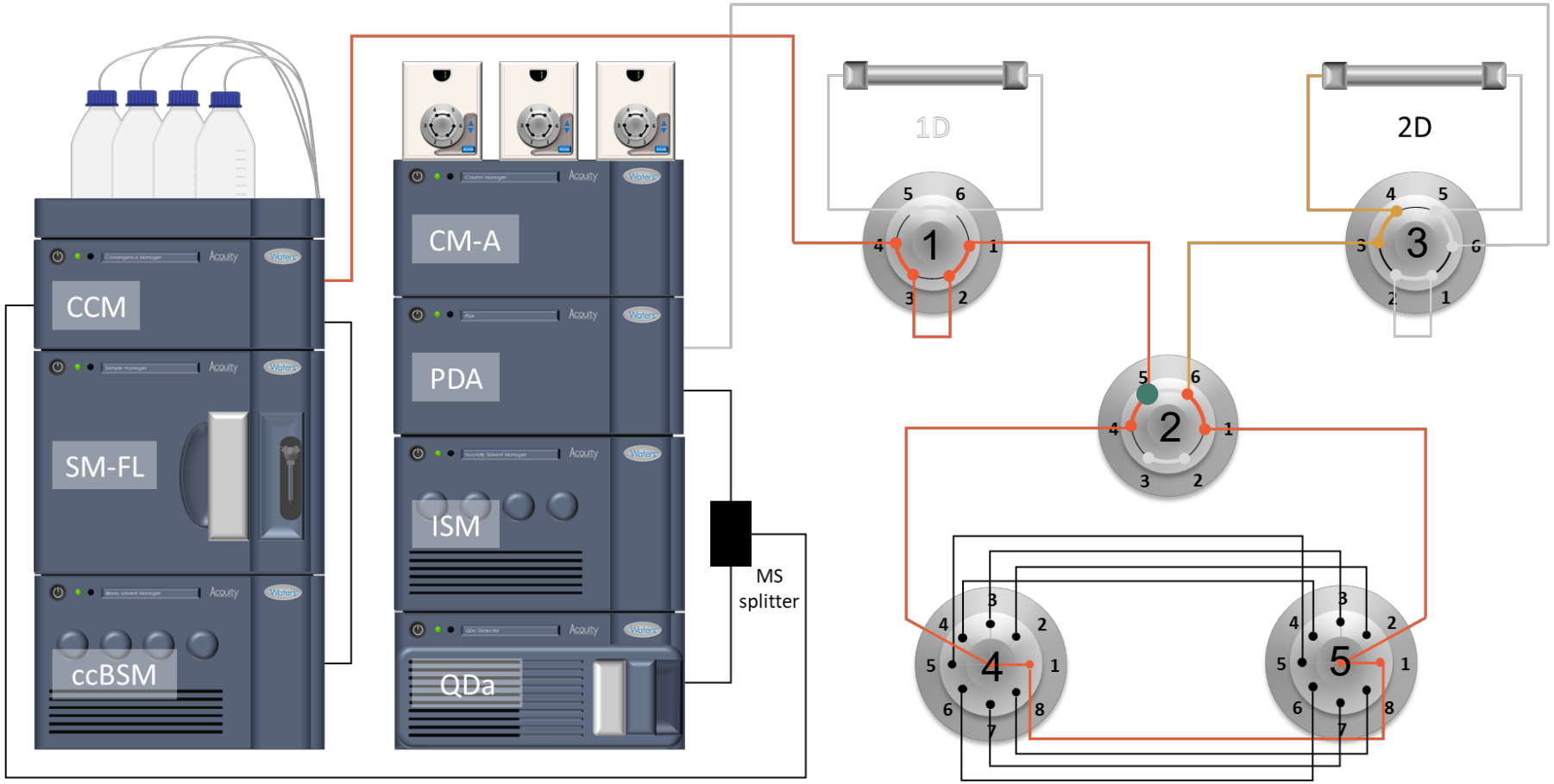
2D system



The mobile phase of dim2 bypasses the column of dim1

2D system – unload loop in dim2

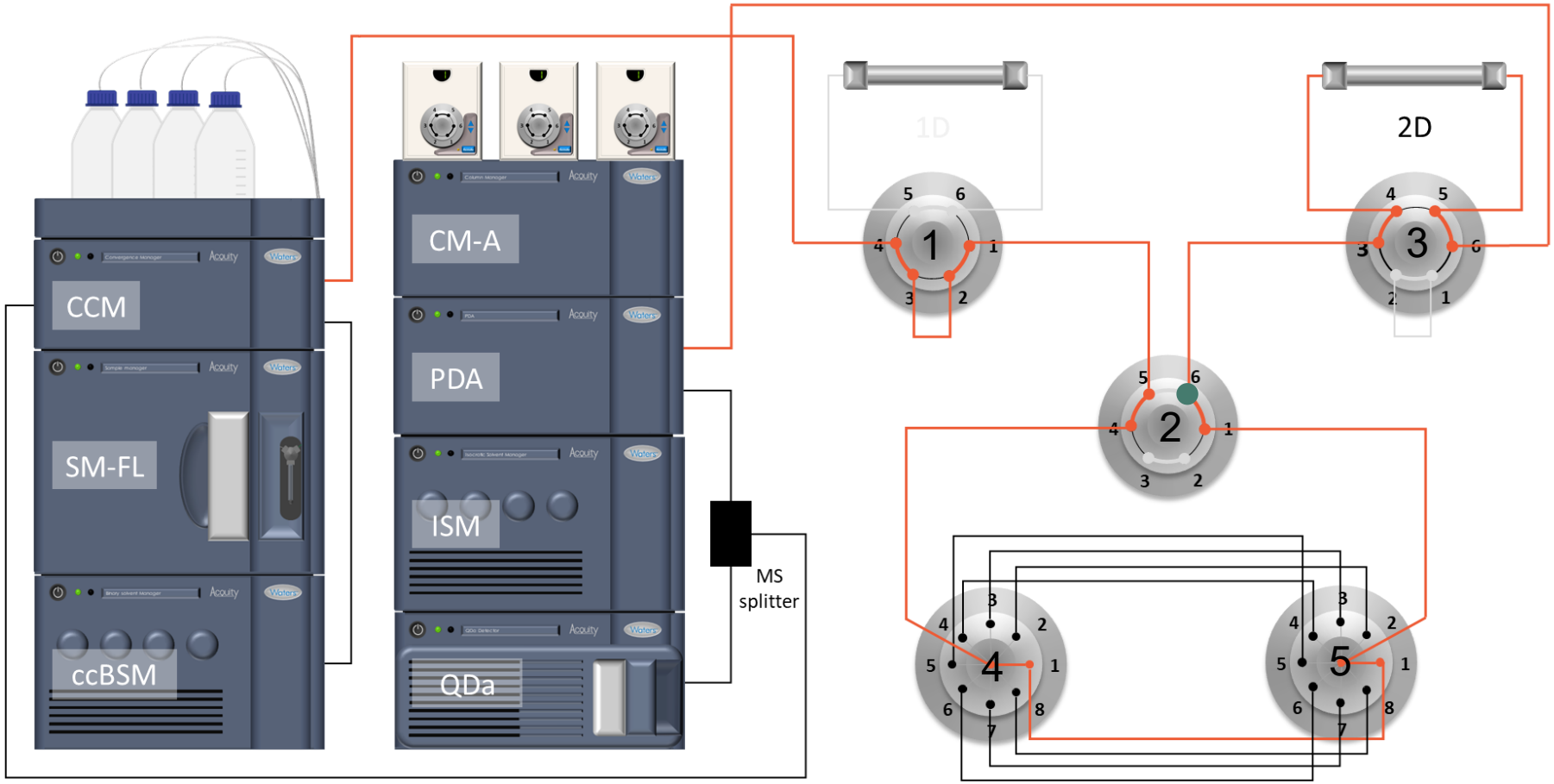
2D system



The mobile phase pushes the contents of the loop into the dim2 column

2D system – unload loop in dim2

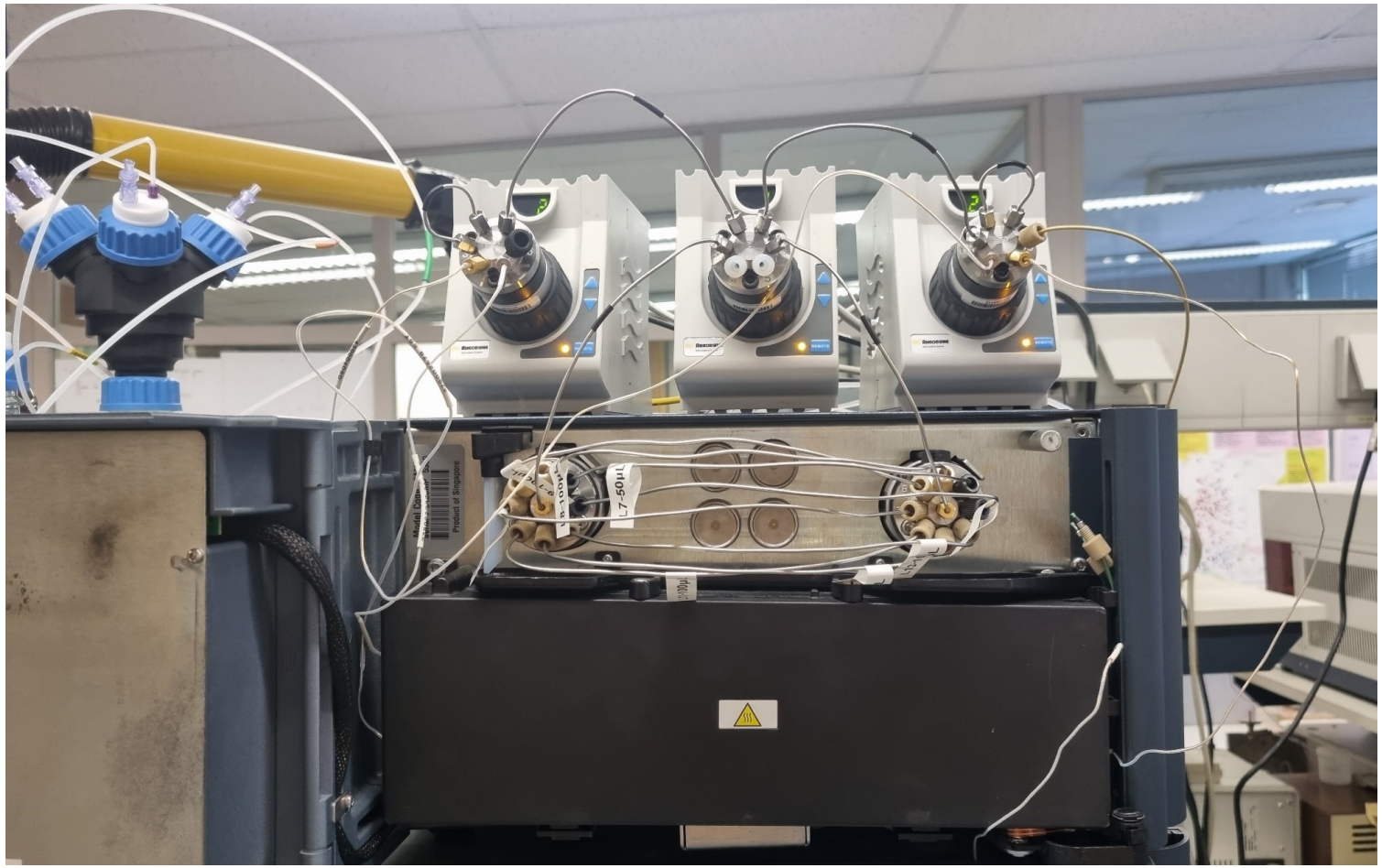
2D system



The analysis continues all the way to the detector

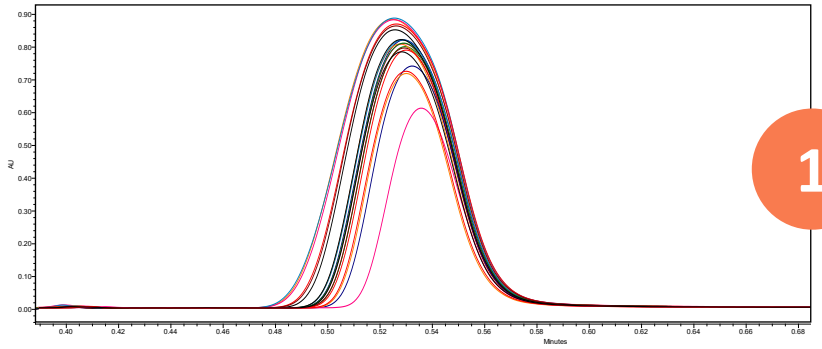
2D system – multiple heart-cut

2D system



Difficulties

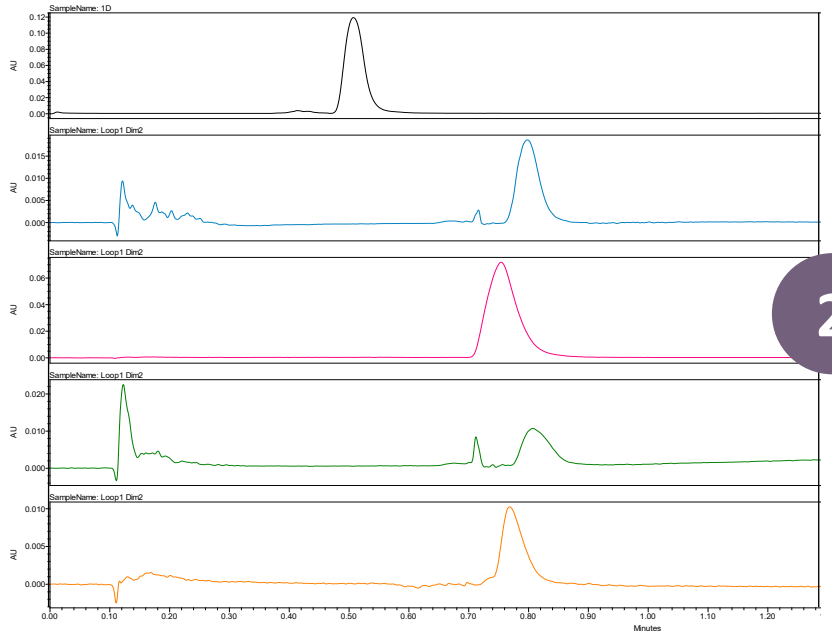
Difficulties



1

Leaks

On each of the ferrules, presence of micro-leaks



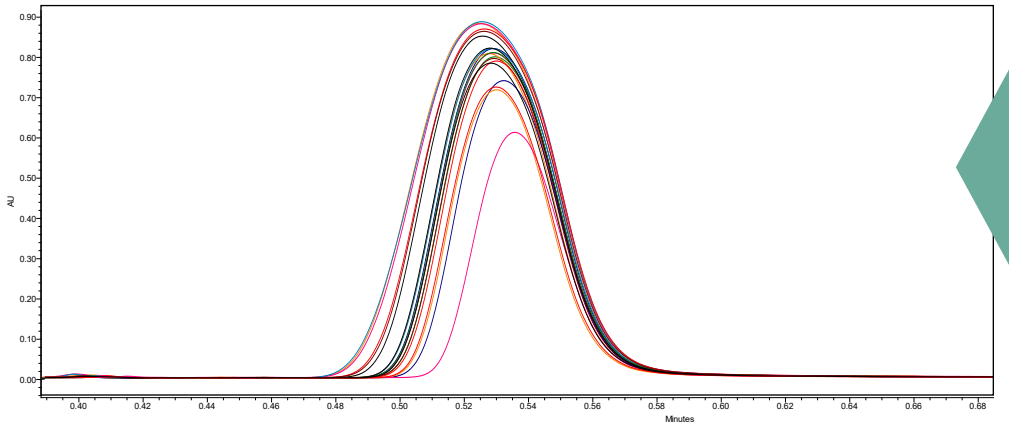
2

Repeatability

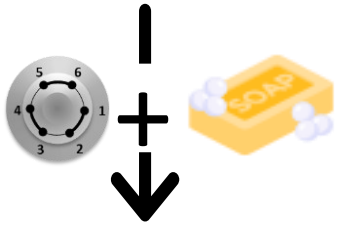
From one loop to another step we did not recover the same quantity

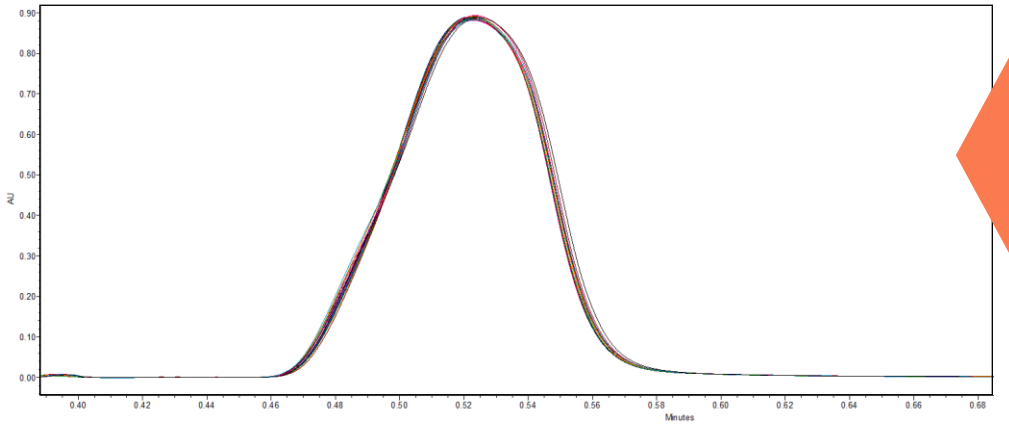
Difficulties

Difficulties



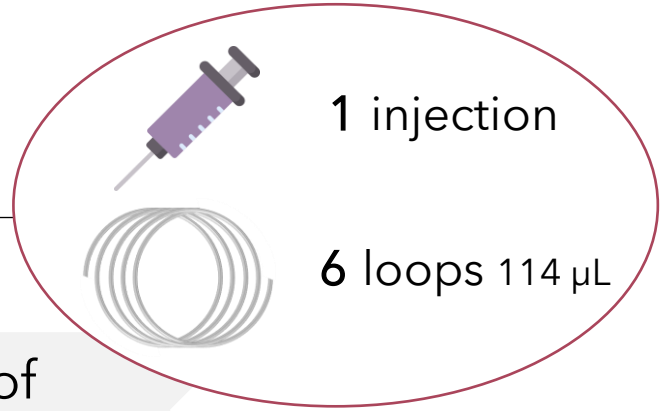
Variation in peak intensity over 20 replicates





Repeatable on 20 replicates with RSD < 2%

Optimization – time of switch



Load more extensively before and after peaks of interest by loading 6 loops

Results

Sample

Peels

Extraction: 1 g of sample + 15 mL of EtOH (maceration in US bath for 1 h)

Injection volume: 5 µL

1st dimension

Achiral method

Column: Acquity UPC² Torus DEA (100 x 3.0 mm – 1.7 µm)

Co-solvent: 20-100% MeOH + 0.1% MSA

Flow rate: 1.7-0.6 mL/min

BPR: 150-110 bar

Oven: 30°C

Detection: 280 nm

2nd dimension

Chiral method

Column: Chiralpak IB (150 x 4.6 mm – 3.0 µm)

Co-solvent: 15-50% MeOH + 0.1% MSA

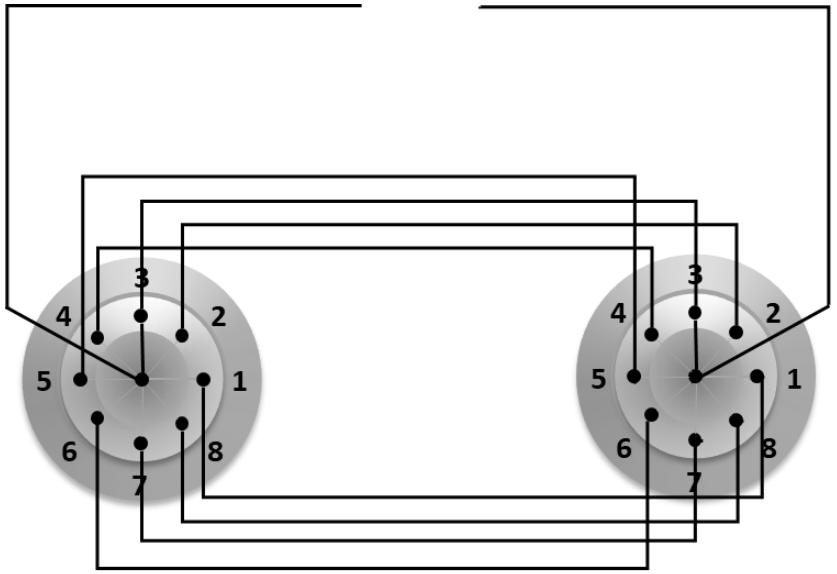
Flow rate: 2.0 mL/min

BPR: 150 bar

Oven: 30°C

Detection: 280 nm

Optimization – time of switch

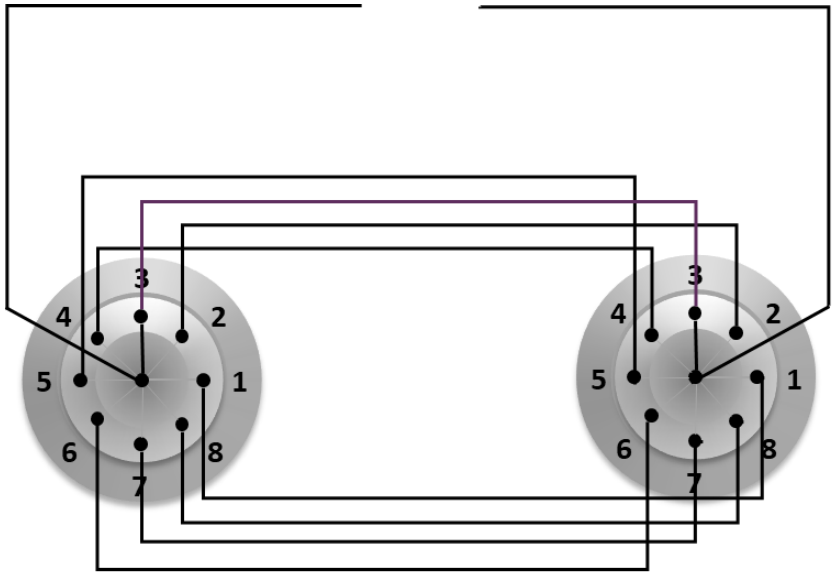


Results

Time of switch (min)	
5.51	
5.62	← Neohesperidin t_R = 5.72 min
5.73	
5.84	
5.95	← Naringin t_R = 5.98 min
6.06	

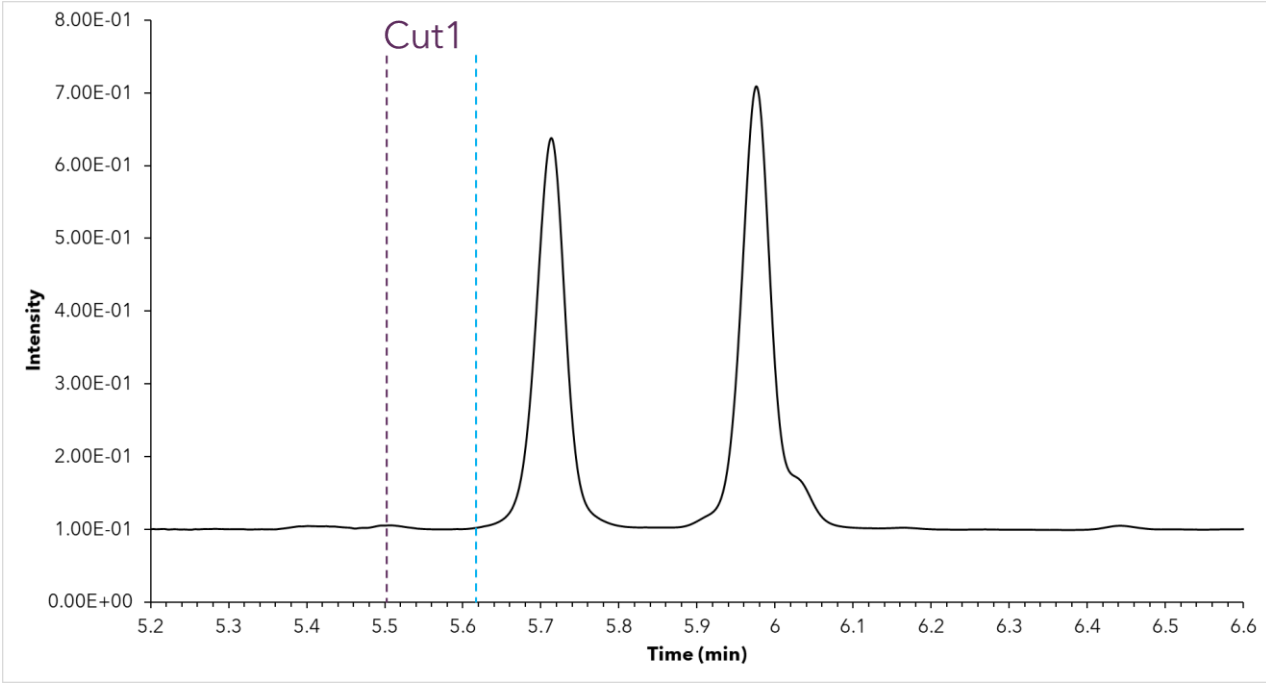
- Retention time
- Eluting composition
- Delay between column 1 and PDA
- Loop volume

Optimization – time of switch

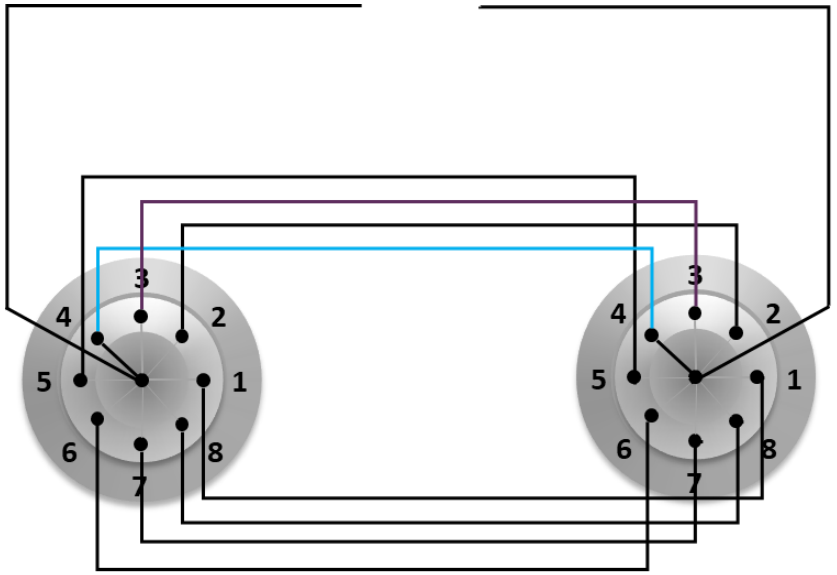


Results

	Time of switch (min)	
Cut1	5.51	
	5.62	← Neohesperidin t_R = 5.72 min
	5.73	
	5.84	
	5.95	← Naringin t_R = 5.98 min
	6.06	

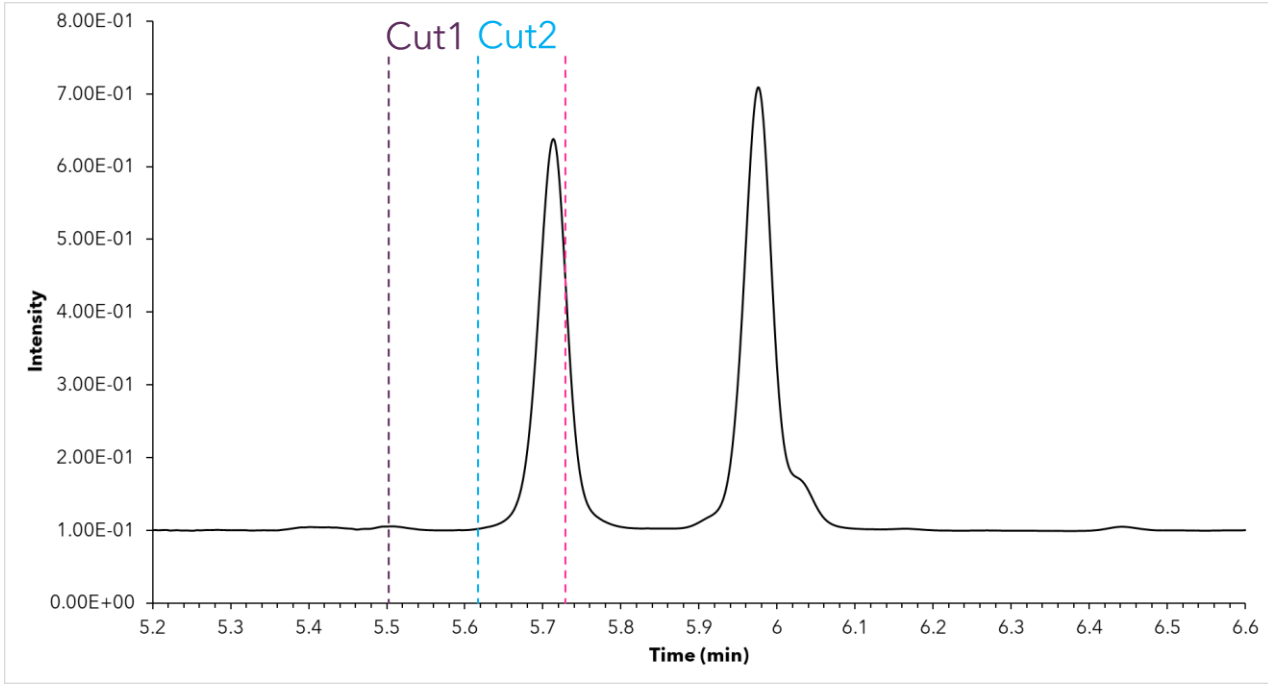


Optimization – time of switch



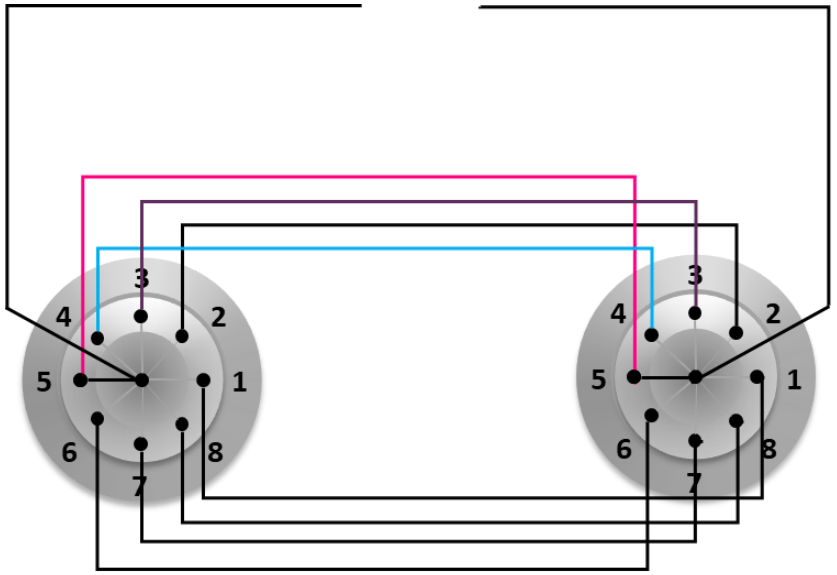
Results

	Time of switch (min)	
Cut1	5.51	
Cut2	5.62	← Neohesperidin t_R = 5.72 min
	5.73	
	5.84	
	5.95	← Naringin t_R = 5.98 min
	6.06	

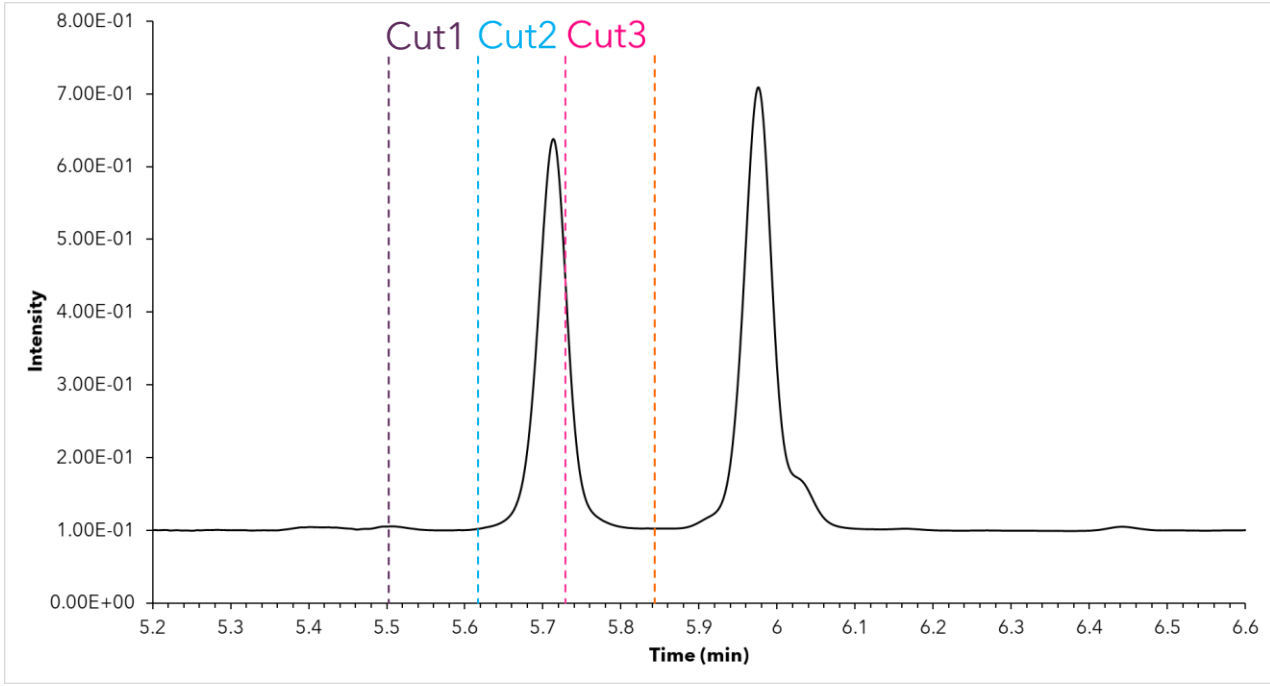


Optimization – time of switch

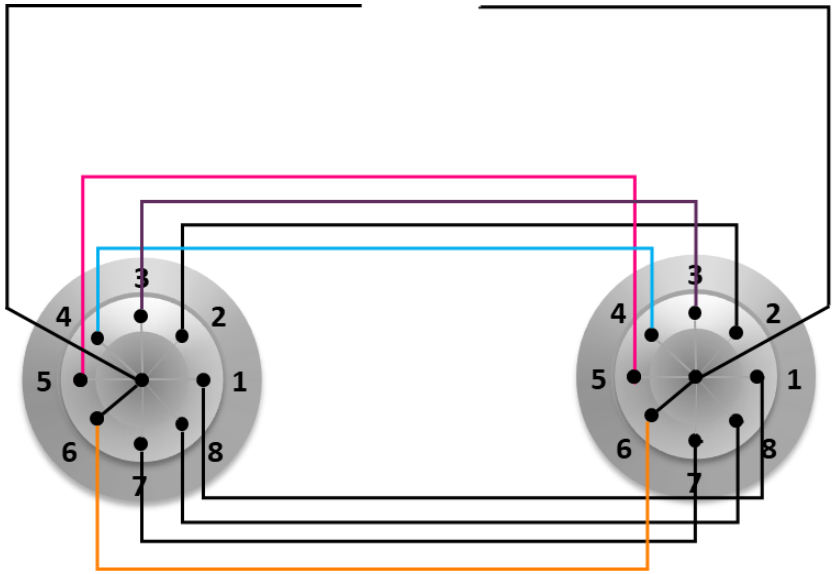
Results



	Time of switch (min)	
Cut1	5.51	
Cut2	5.62	
Cut3	5.73	← Neohesperidin t_R = 5.72 min
	5.84	
	5.95	← Naringin t_R = 5.98 min
	6.06	

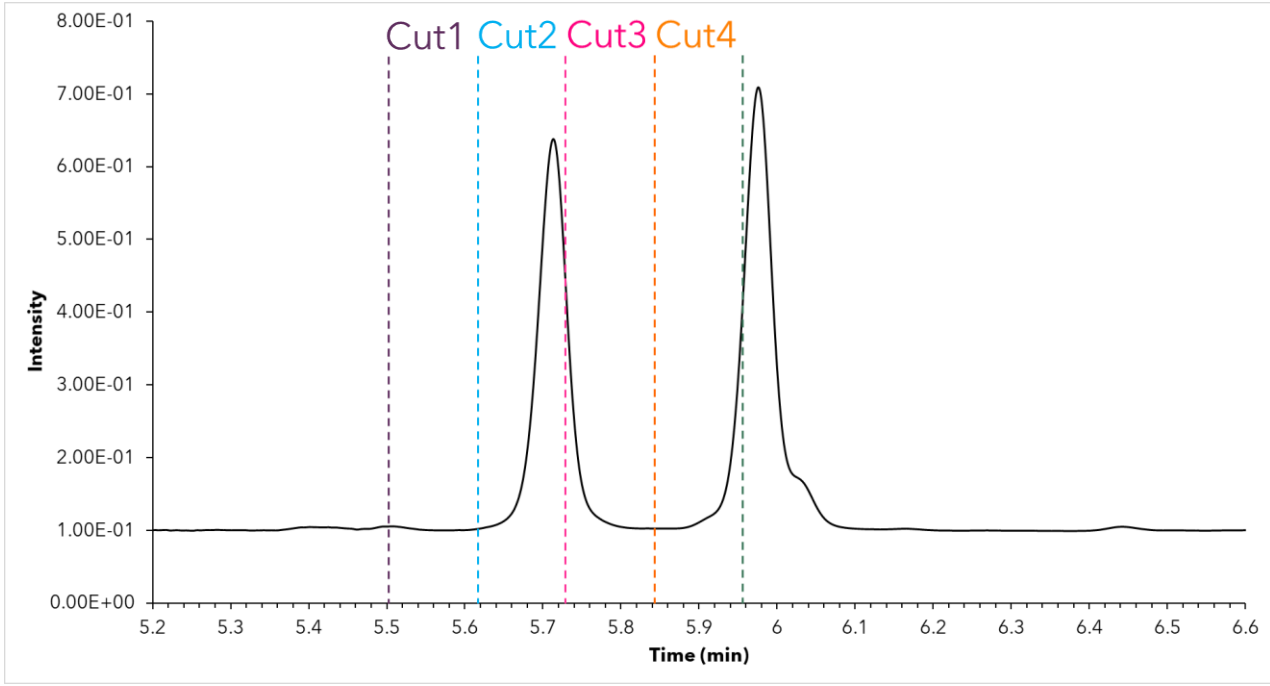


Optimization – time of switch



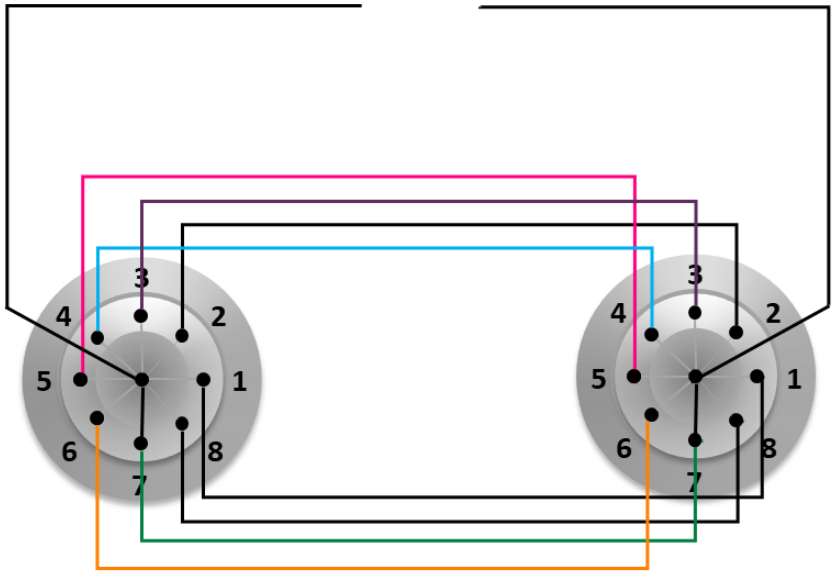
Results

	Time of switch (min)	
Cut1	5.51	
Cut2	5.62	← Neohesperidin t_R = 5.72 min
Cut3	5.73	
Cut4	5.84	
	5.95	← Naringin t_R = 5.98 min
	6.06	



Optimization – time of switch

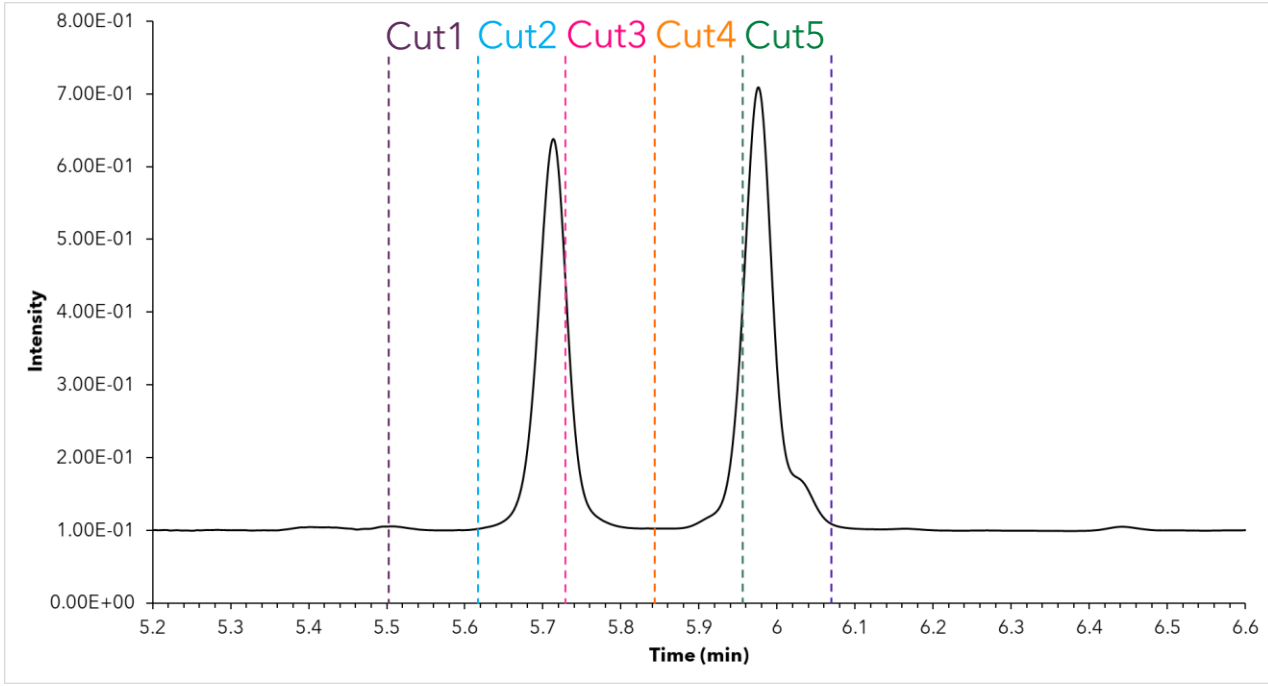
Results



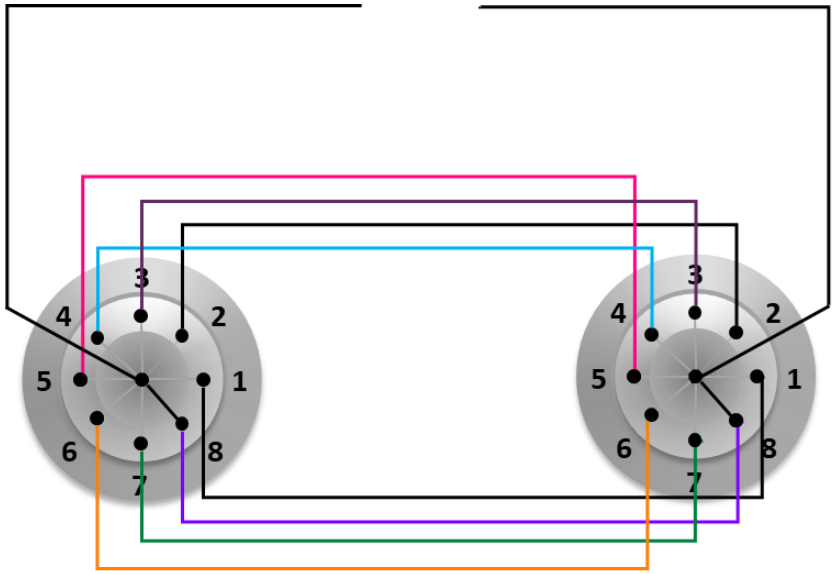
	Time of switch (min)
Cut1	5.51
Cut2	5.62
Cut3	5.73
Cut4	5.84
Cut5	5.95
	6.06

← Neohesperidin
t_R = 5.72 min

← Naringin
t_R = 5.98 min



Optimization – time of switch

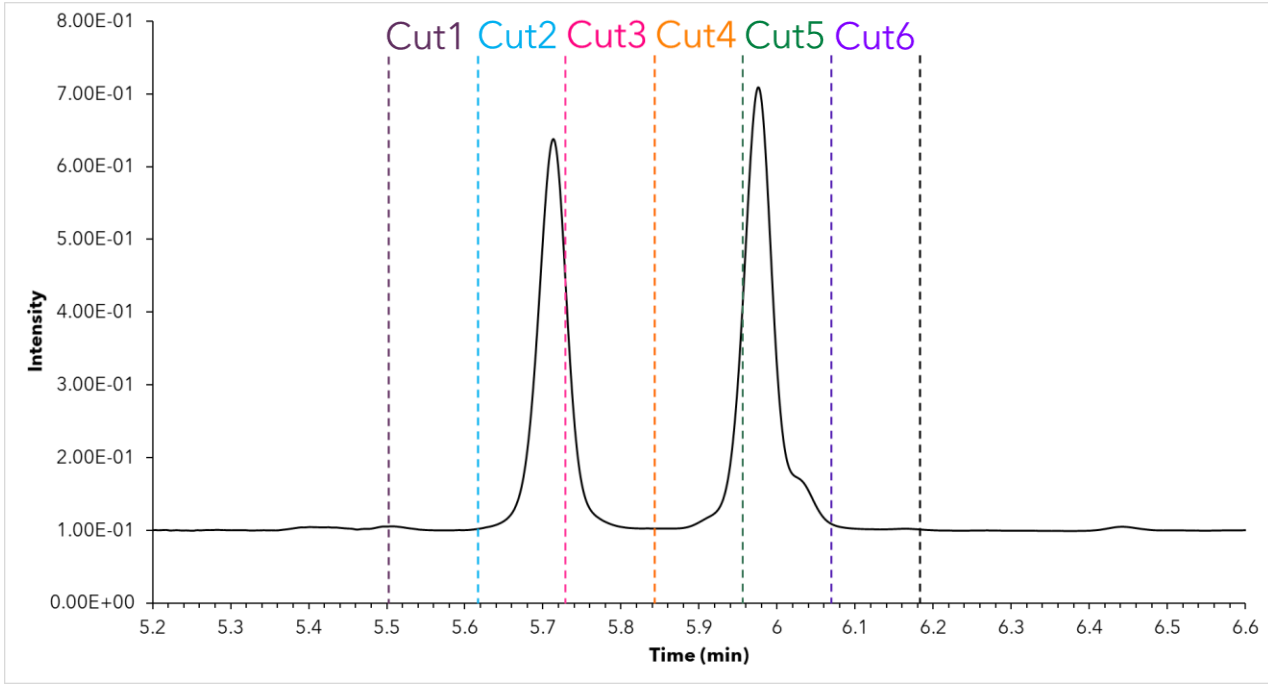


Results

	Time of switch (min)
Cut1	5.51
Cut2	5.62
Cut3	5.73
Cut4	5.84
Cut5	5.95
Cut6	6.06

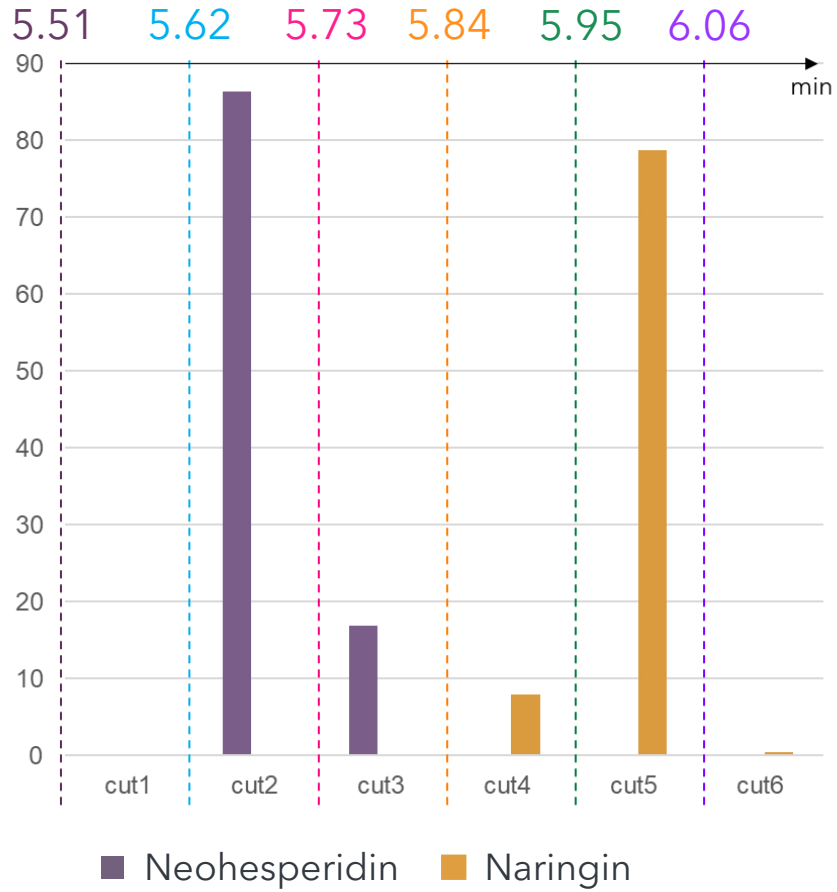
← Neohesperidin
t_R = 5.72 min

← Naringin
t_R = 5.98 min



Optimization – transfer rate

Results



$$\text{Transfert rate} = \frac{\text{Sum area of dim2 peaks}}{\text{Area of peak interest}}$$

86% recovered for Neohesperidin in cut2
78% recovered for Naringin in cut5

No overlapping of sampling times

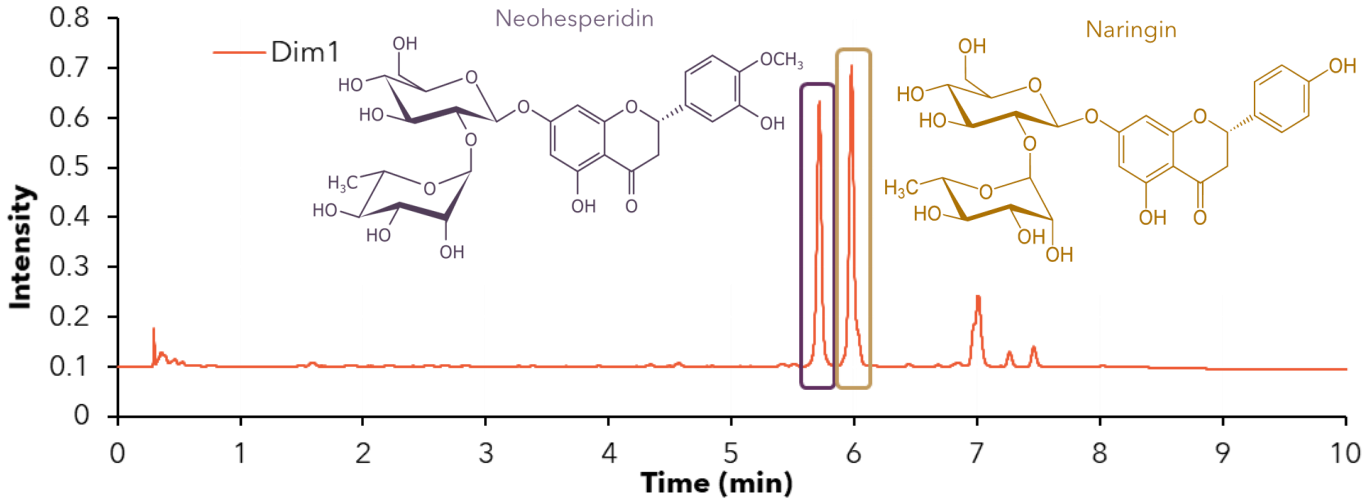
Results – UV

Results

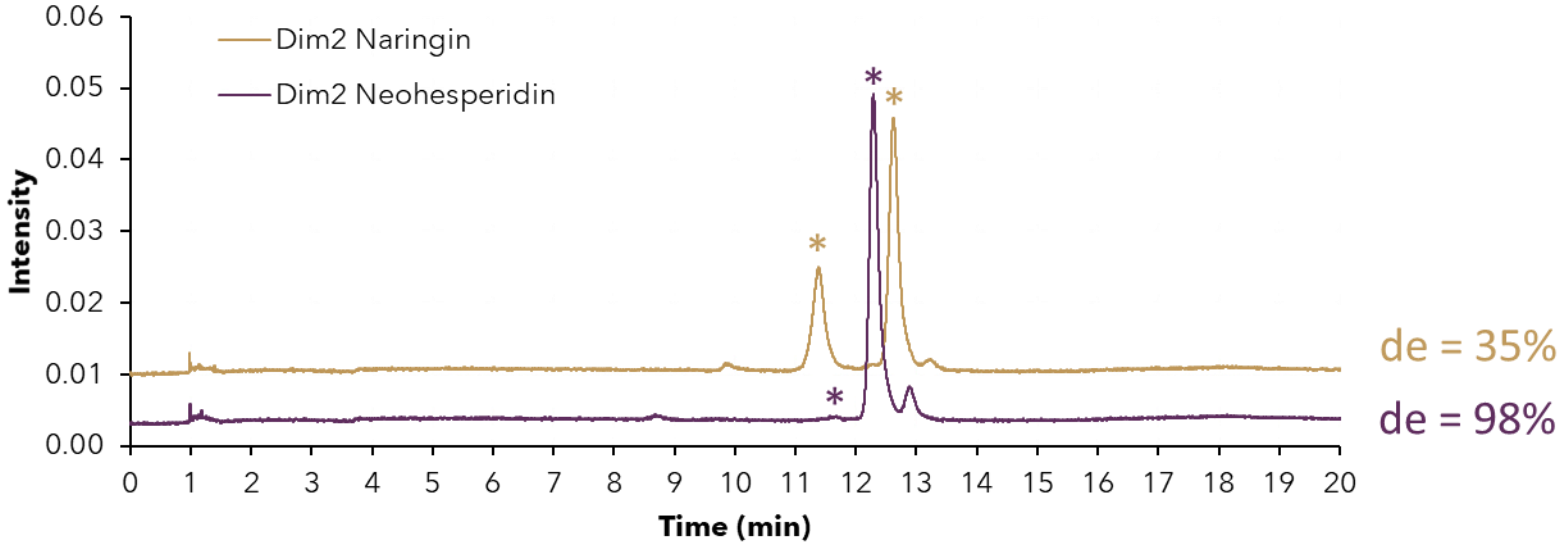
dim1

dim2

First dimension

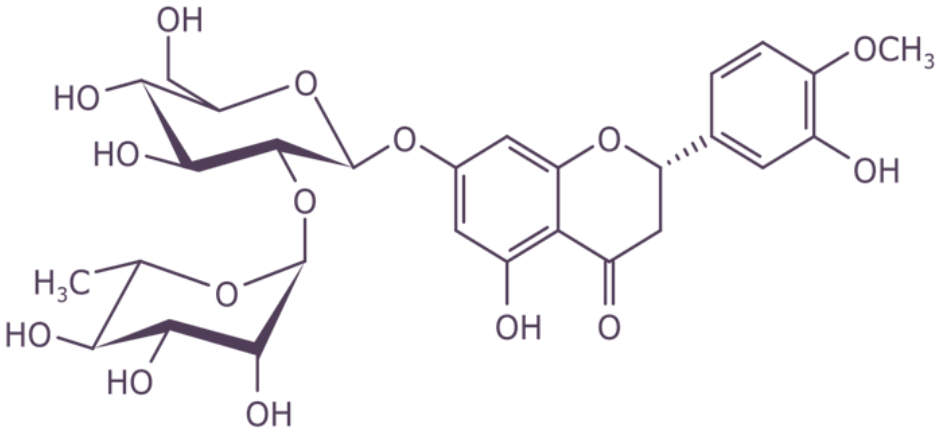


Second dimension

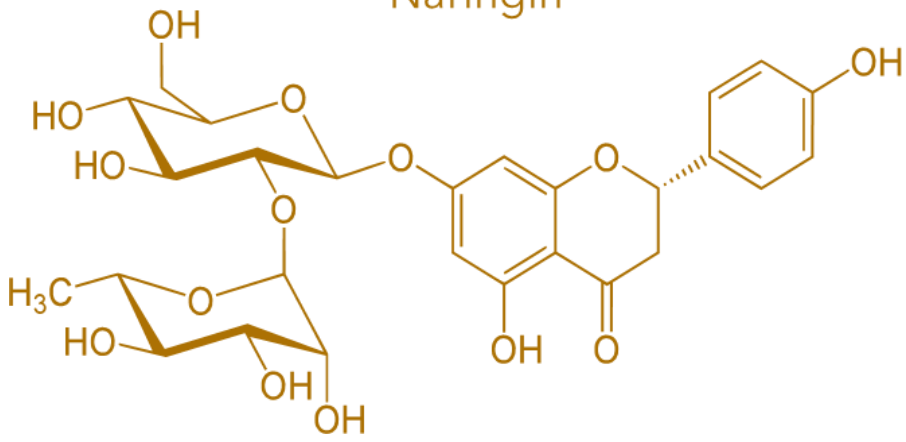


Results – MS

Neohesperidin



Naringin

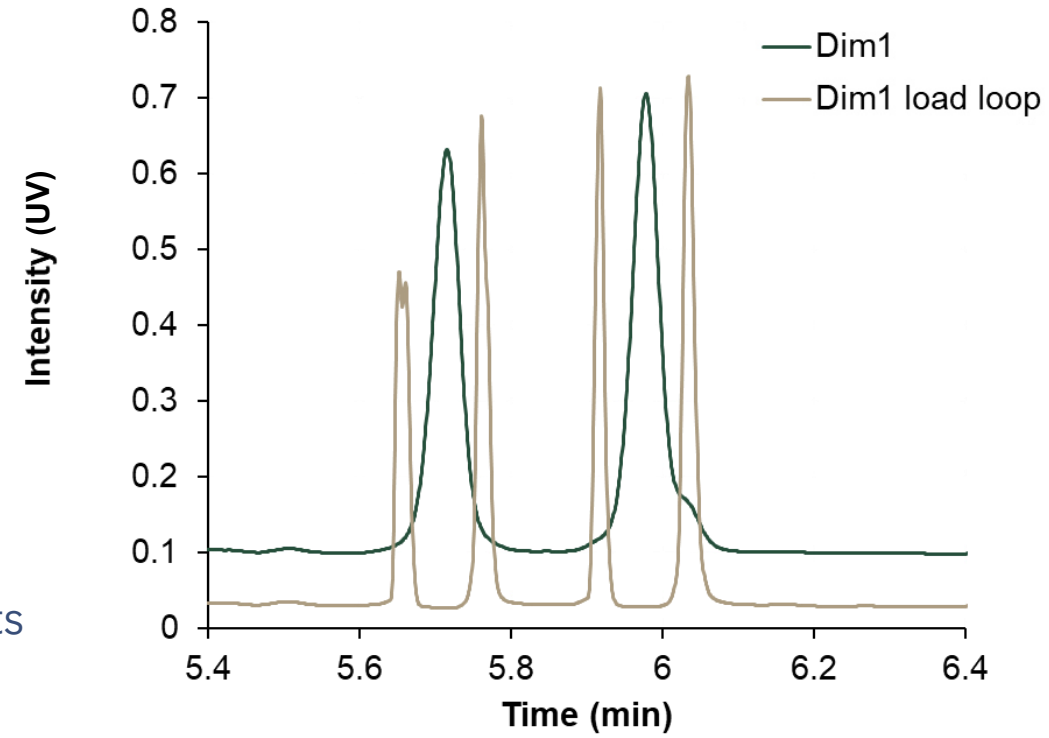


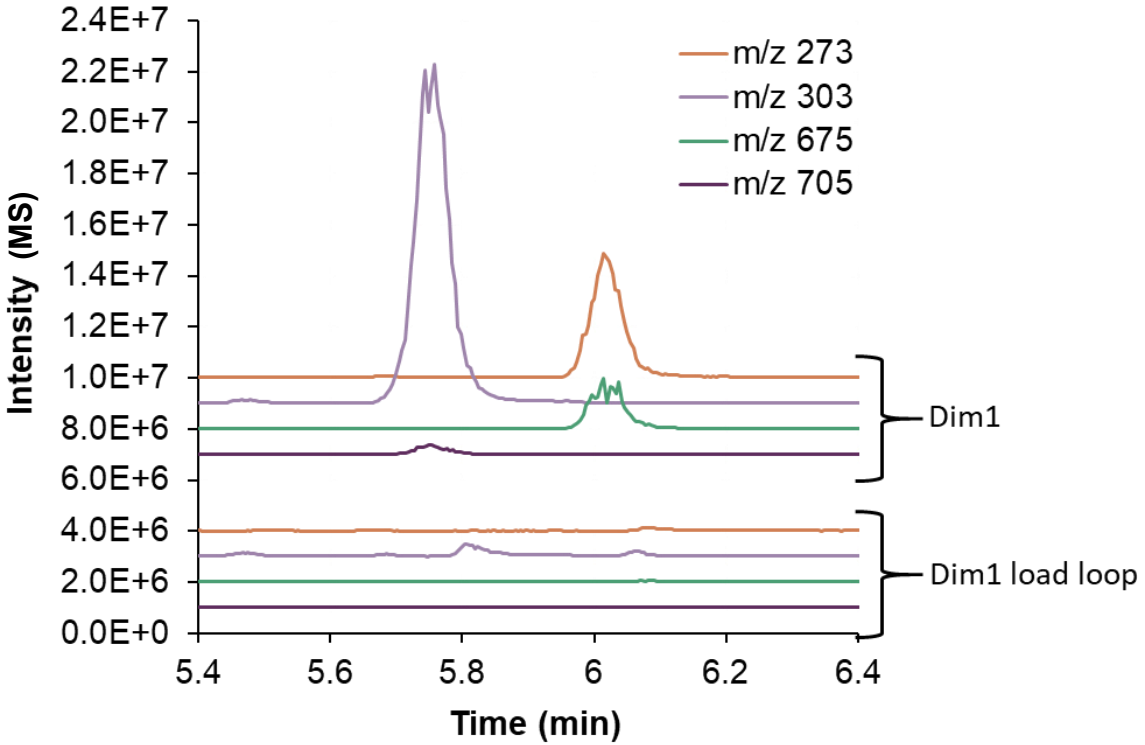
Results

	MW (g/mol)	$[M + MSA]^-$	$[M - C_{12}H_{22}O_9 + H]^+$ (aglycone)
Neohesperidin	610.56	*m/z* 705	*m/z* 303
Naringin	580.54	*m/z* 675	*m/z* 273

Results - MS

Results





Presence of target compounds in dim1.
When the loops are loaded, the mass signal corresponding to the target compounds is absent.

Results – objectives

Results

Determining the diastereoisomeric excesses of flavonoids

Sample

Bitter orange parts

Extraction: 1 g of sample + 15 mL of EtOH (maceration in US bath for 1 h)

Injection volume: 5 µL

1st dimension

Achiral method

Column: Acquity UPC² Torus DEA (100 x 3.0 mm – 1.7 µm)

Co-solvent: 20-100% MeOH + 0.1% MSA

Flow rate: 1.7-0.6 mL/min

BPR: 150-110 bar

Oven: 30°C

Detection: 280 nm

2nd dimension

Chiral method

Column: Chiralpak IB (150 x 4.6 mm – 3.0 µm)

Co-solvent: 15-50% MeOH + 0.1% MSA

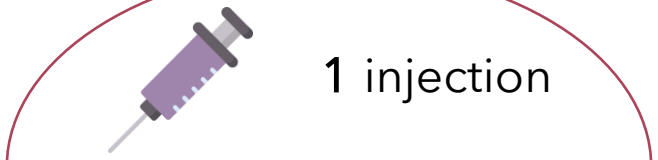
Flow rate: 2.0 mL/min

BPR: 150 bar

Oven: 30°C

Detection: 280 nm

Results – sequence



1 injection

2 loops 114 µL

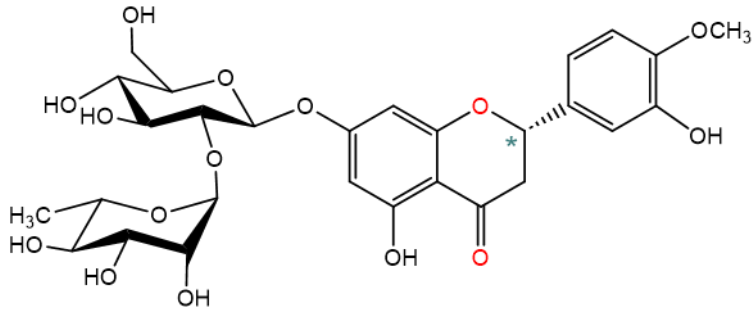
3 h 30
of analysis

Results

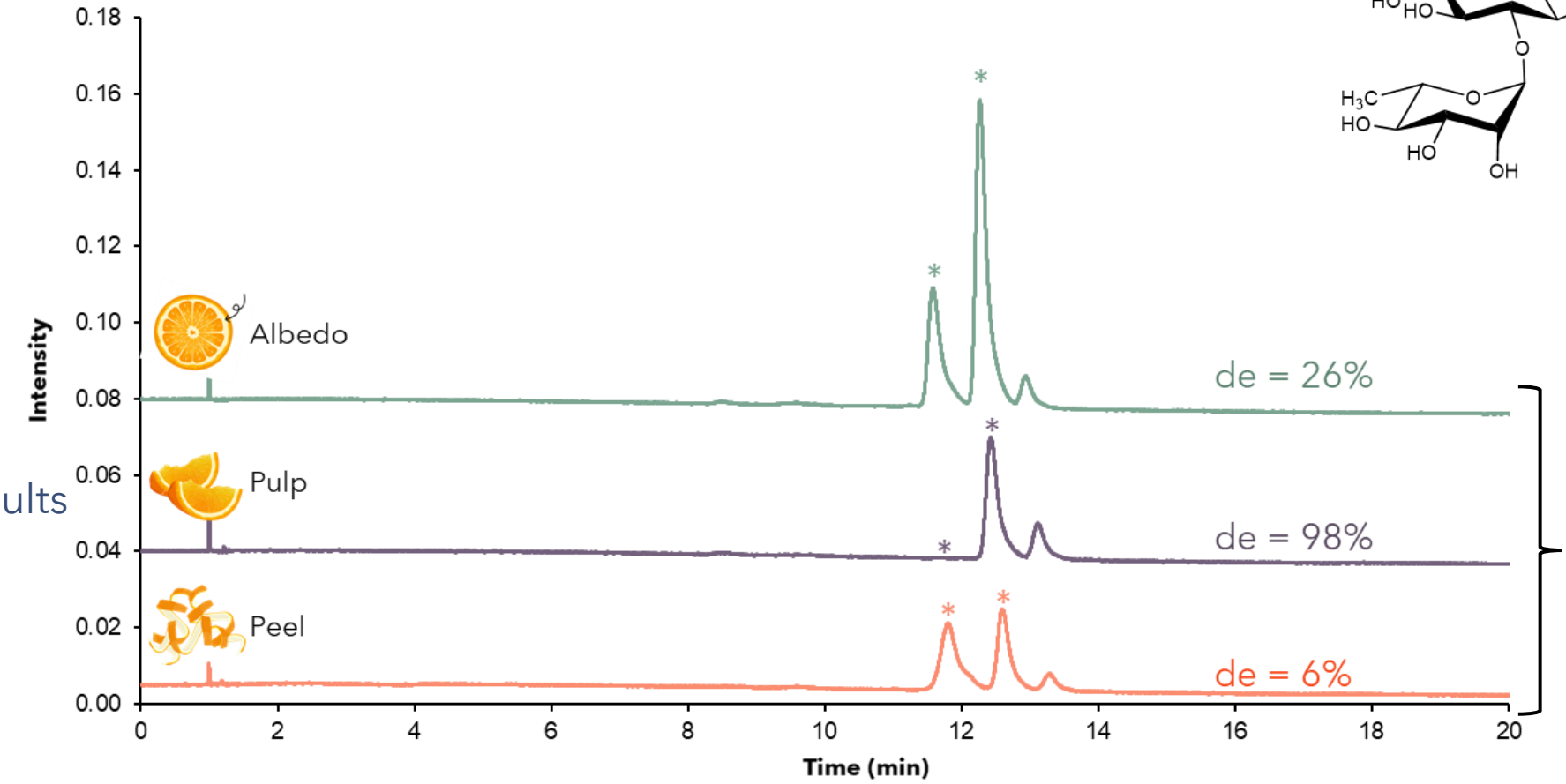
1 min		Change dim2 to dim1
20 min		1st column conditioning
37.5 min	x 3	Blank EtOH
12.5 min		Achiral analysis
12.5 min		Achiral analysis + loading 2 loops
1 min		Change dim1 to dim2
20 min		2nd column conditioning
66 min	x 3	Blank without injection in loop
44 min	x 2	Chiral analysis + unloading loop

Diastereomeric excesses - Neohesperidin

Ripe oranges

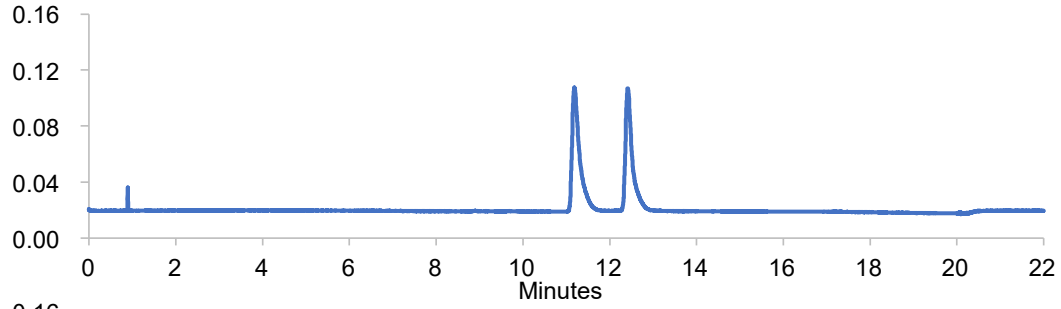


Results

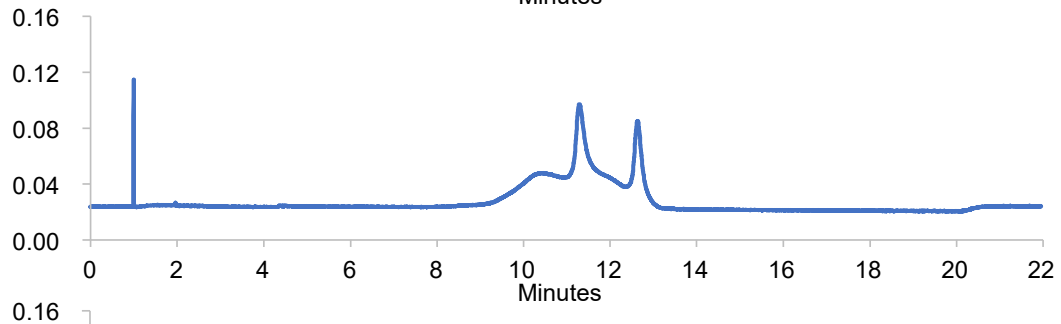


Large variation in diastereomeric excess from one part to another

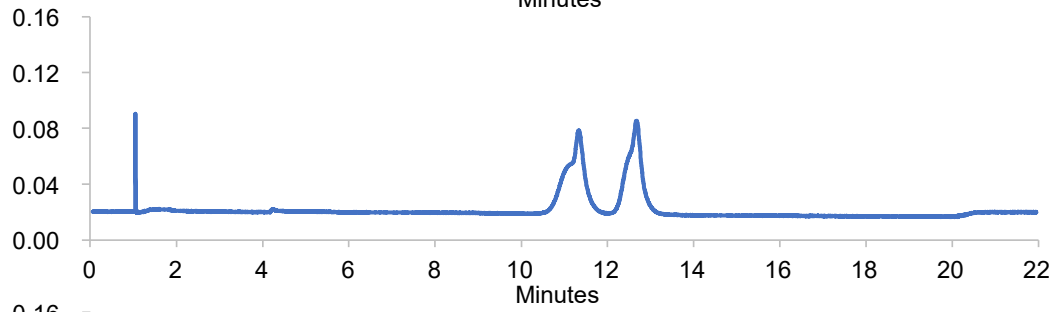
Injection 114 µL



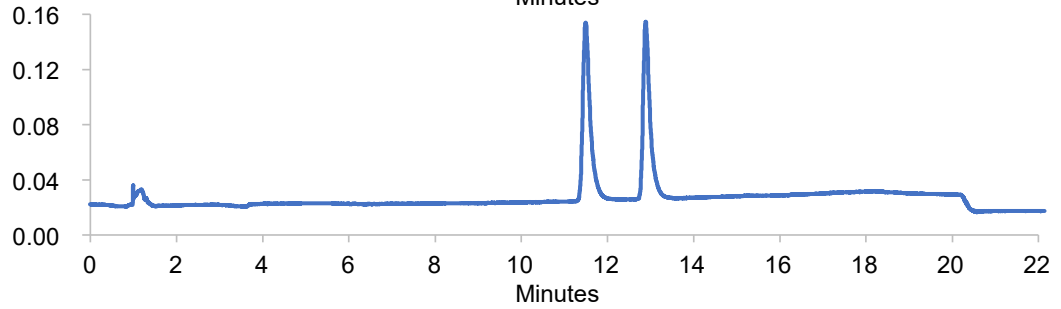
Direct injection 5 µL
Naringin
MeOH



Direct injection 114 µL
Naringin
MeOH



Direct injection 114 µL
Naringin
EtOH/Hept (75/25)



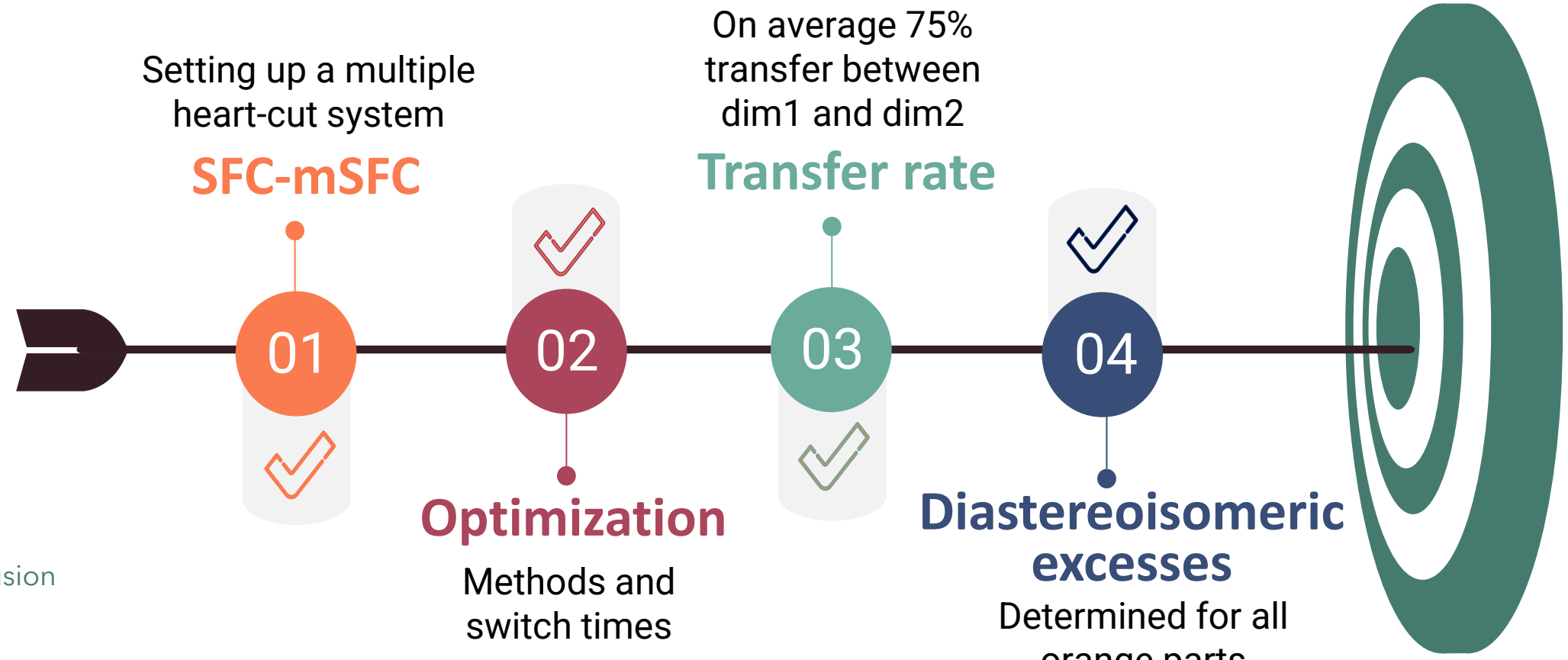
Unload Loop 114 µL
Naringin
MeOH/CO_2 75/25

2nd dimension

Chiral method

Column: Chiralpak IB
(150 x 4.6 mm – 3.0 µm)
Co-solvent: 15-50% MeOH + 0.1% MSA
Flow rate: 2.0 mL/min
BPR: 150 bar
Oven: 30°C
Detection: 280 nm

Conclusion



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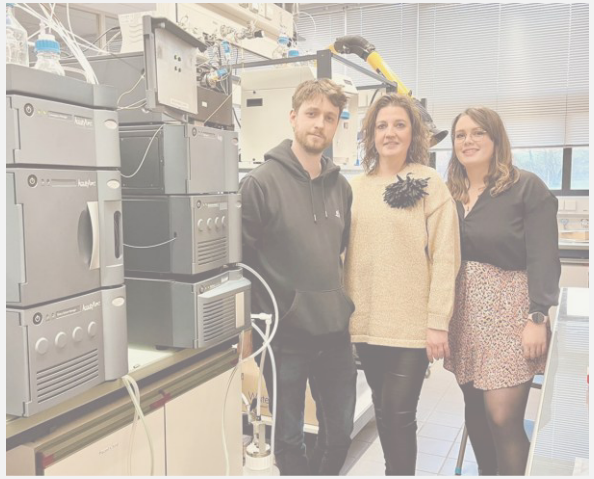

Thank you
For your attention!

Questions ?