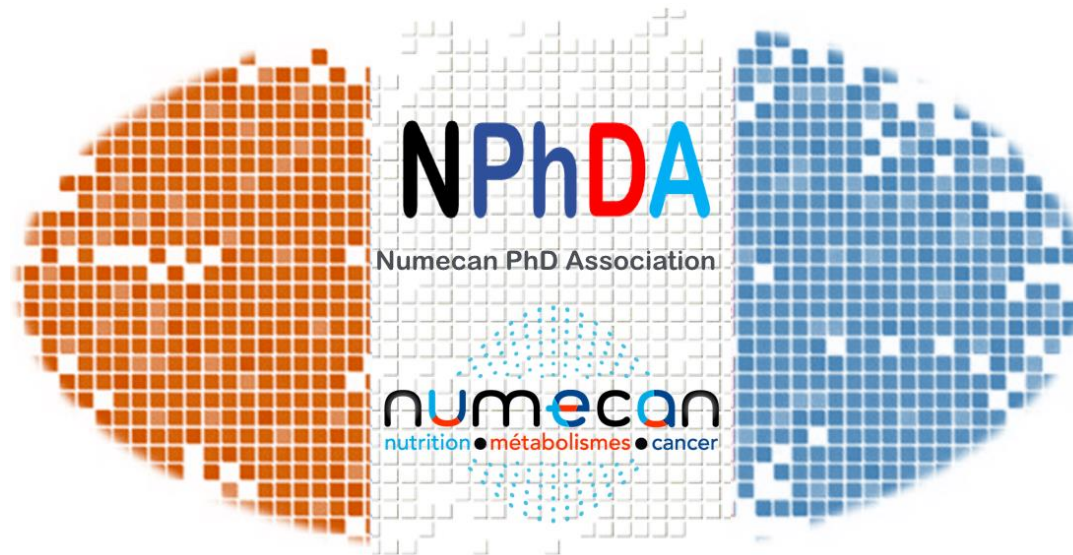


Introduction à l'utilisation de la HPLC

Yann Verres

Réunion technique de NPhDA du Mardi 3 Novembre 2020

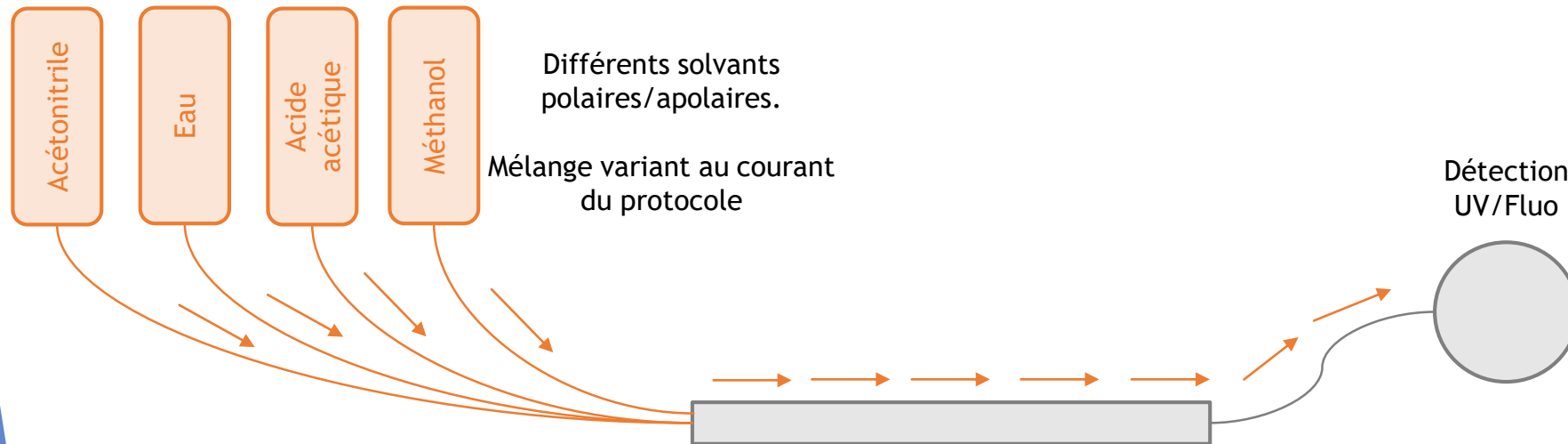


High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée

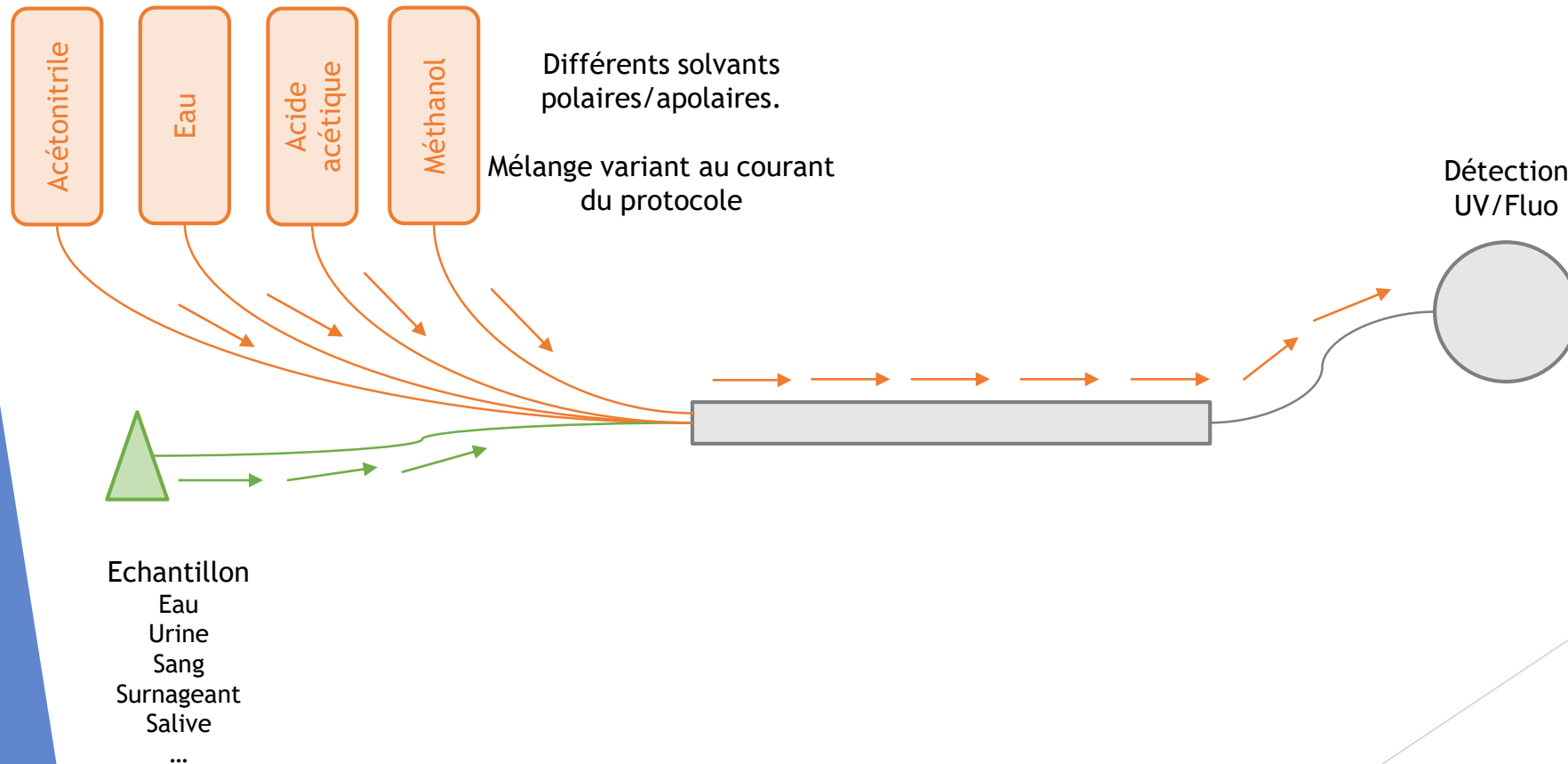
High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée



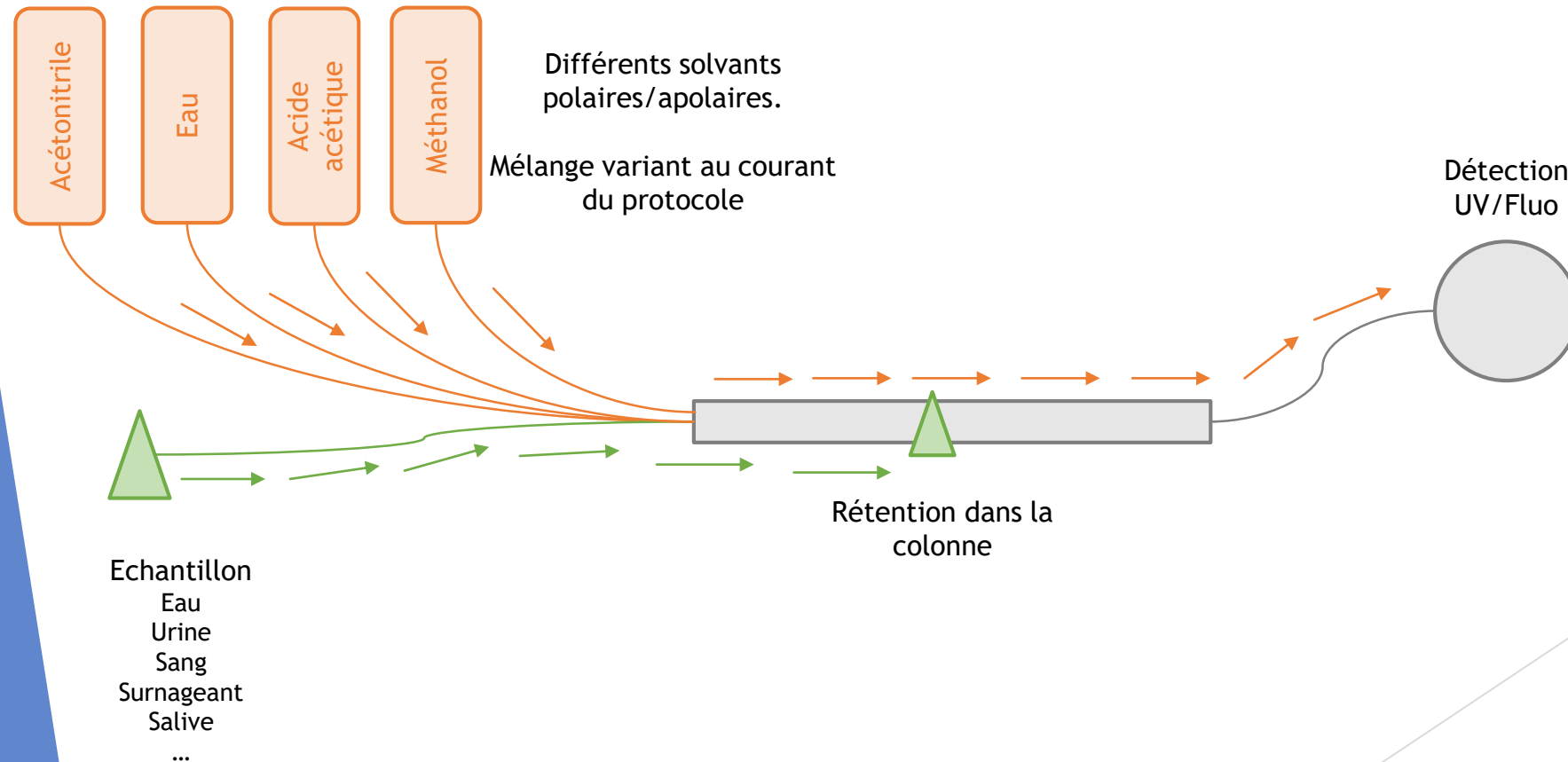
High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée



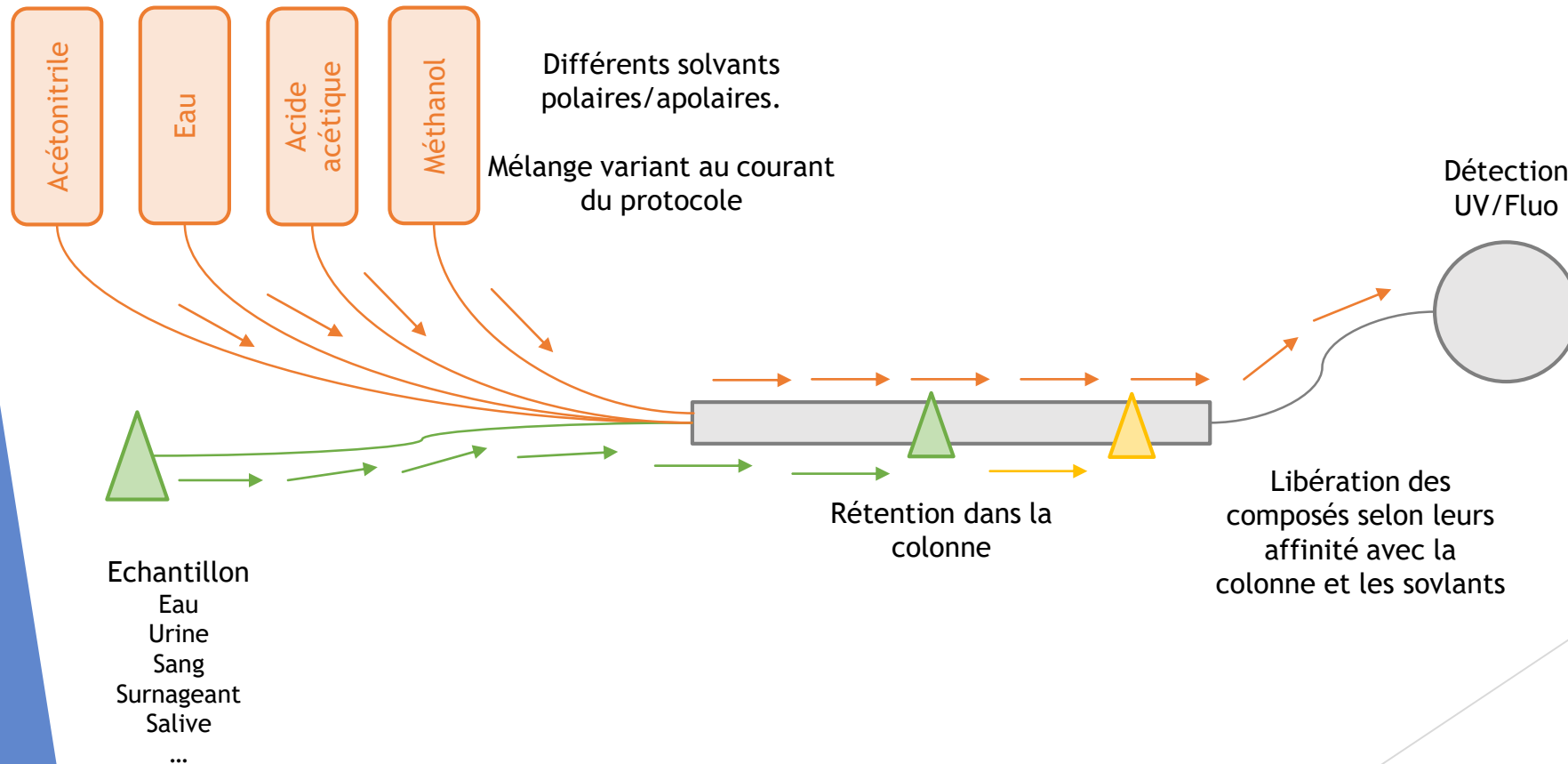
High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée



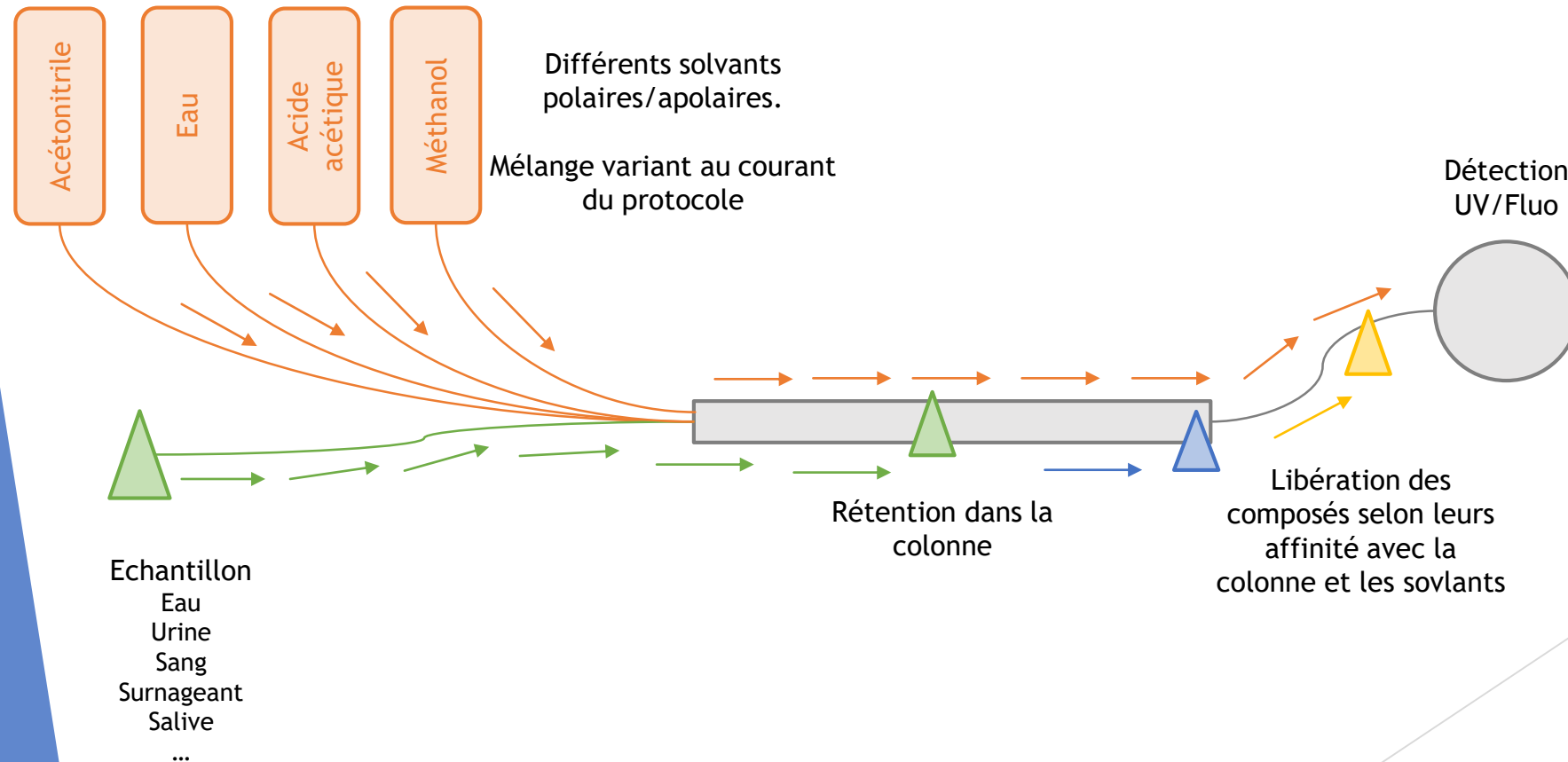
High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée



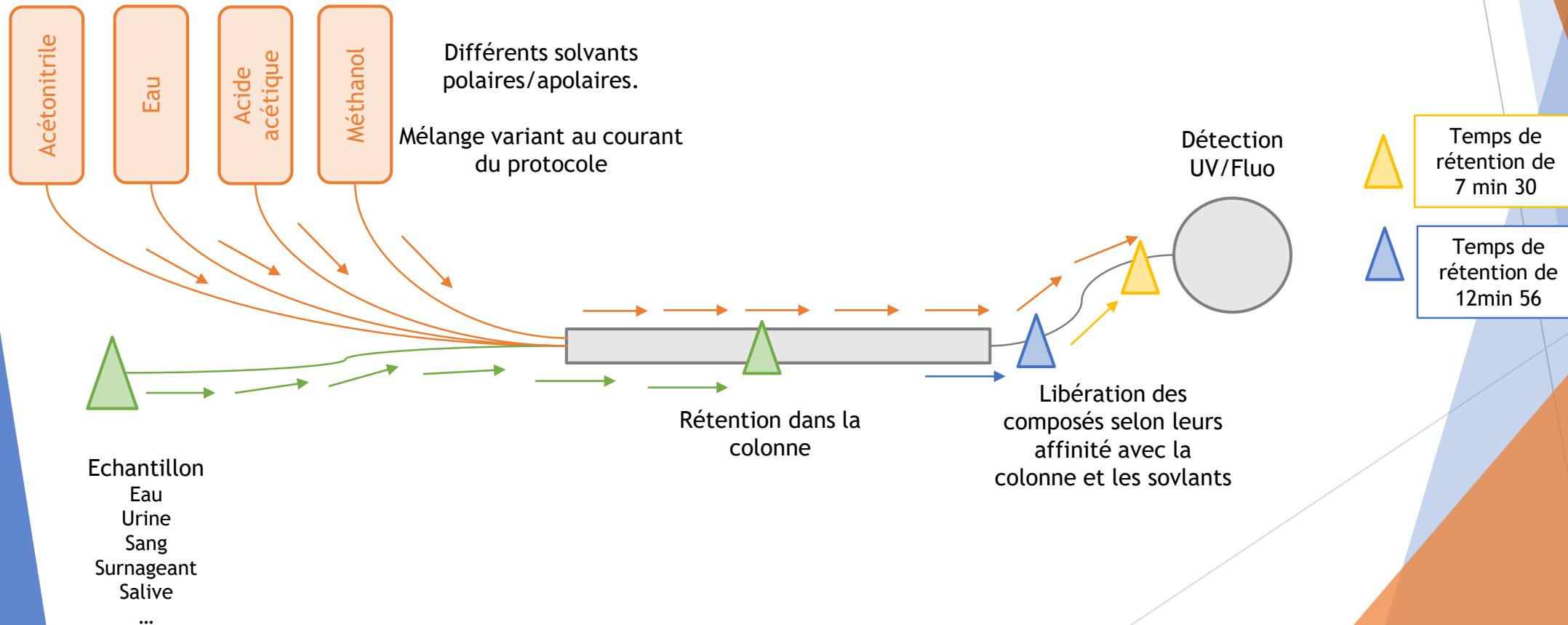
High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée



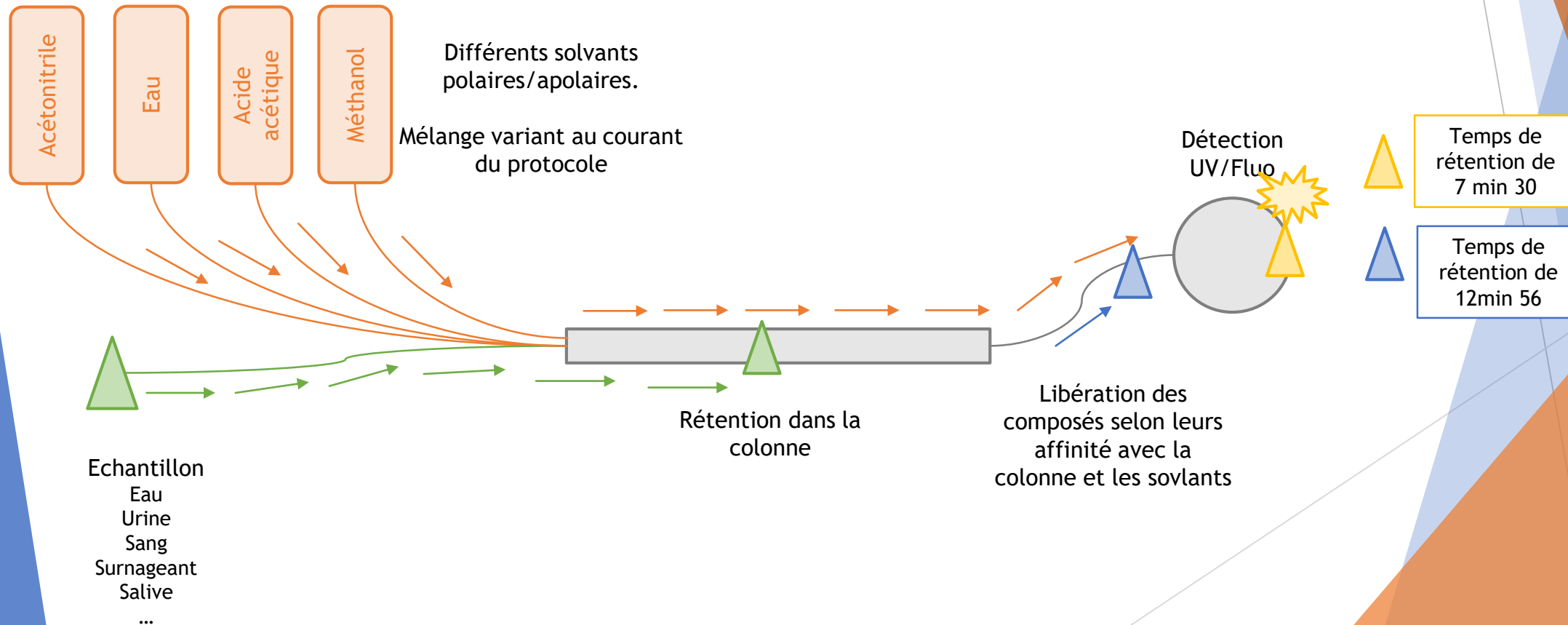
High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée



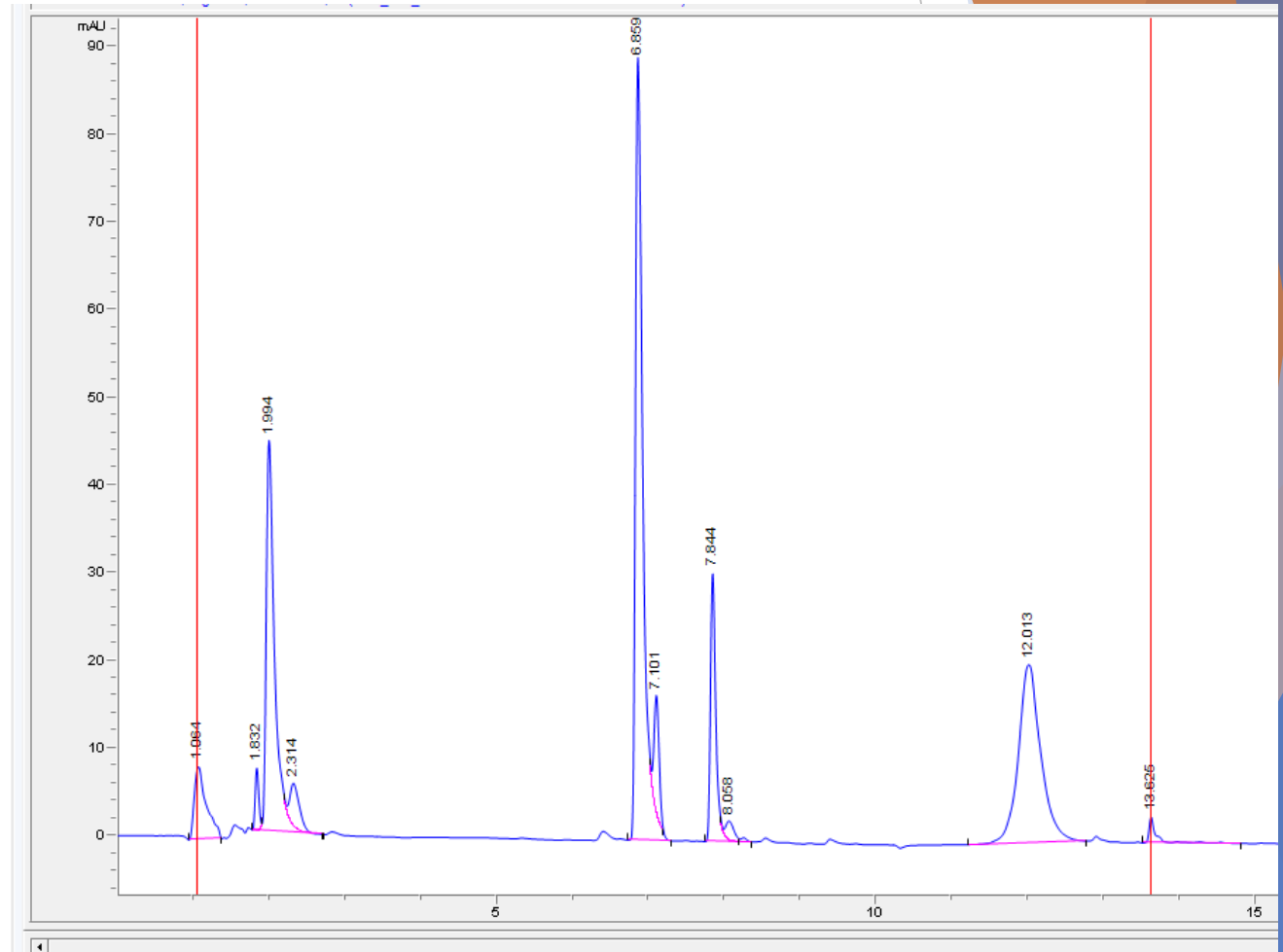
High Performance Liquid Chromatography

Technique de séparer et détecter les différents composants d'une matrice donnée

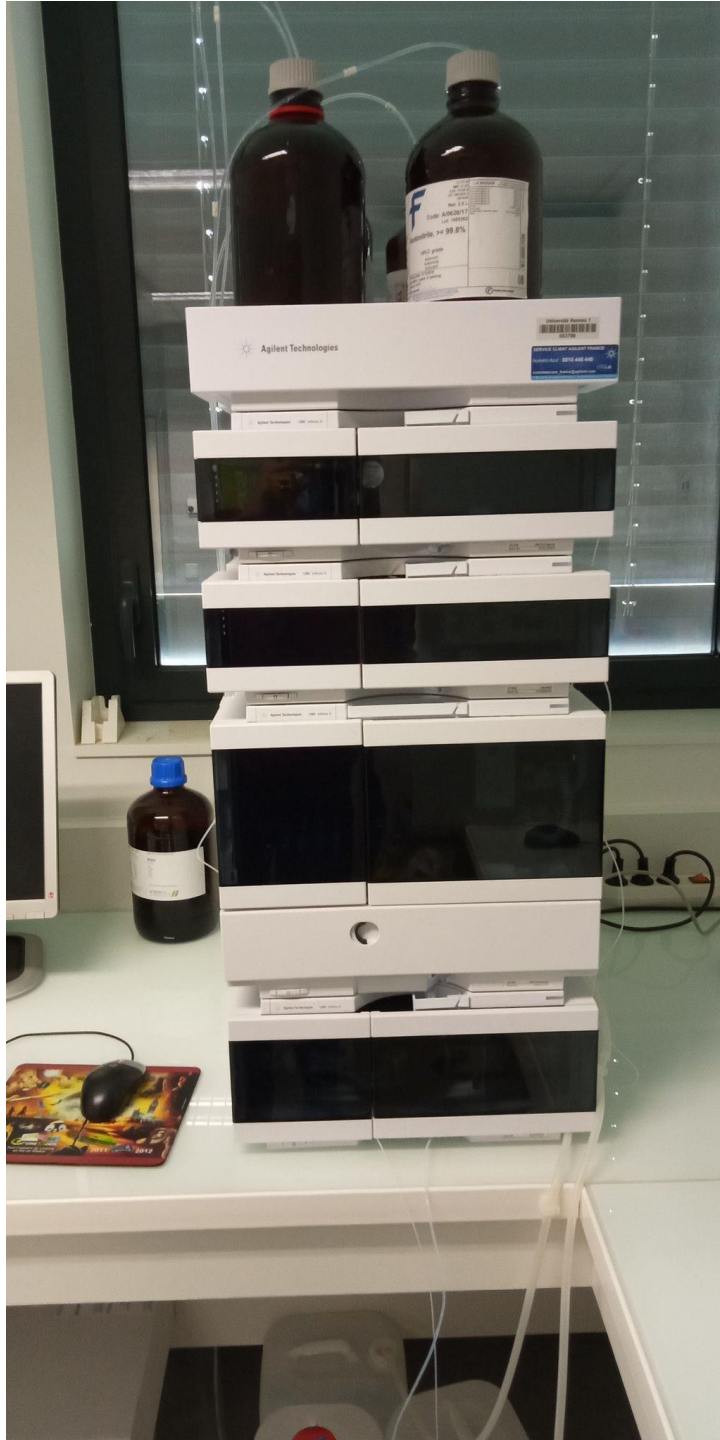


Type de résultats obtenus

- ▶ Profil HPLC, avec des pics correspondant à chaque composé détecté
- ▶ Chaque pic apparaît à un temps de rétention précis, permettant d'identifier des molécules en particulier, si comparé à des standards analytiques
- ▶ Aire sous la courbe permet une quantification du composé, en comparaison à une gamme étalon
- ▶ Les courbes sont superposables, pour comparer l'évolution des quantités d'un échantillon à l'autre

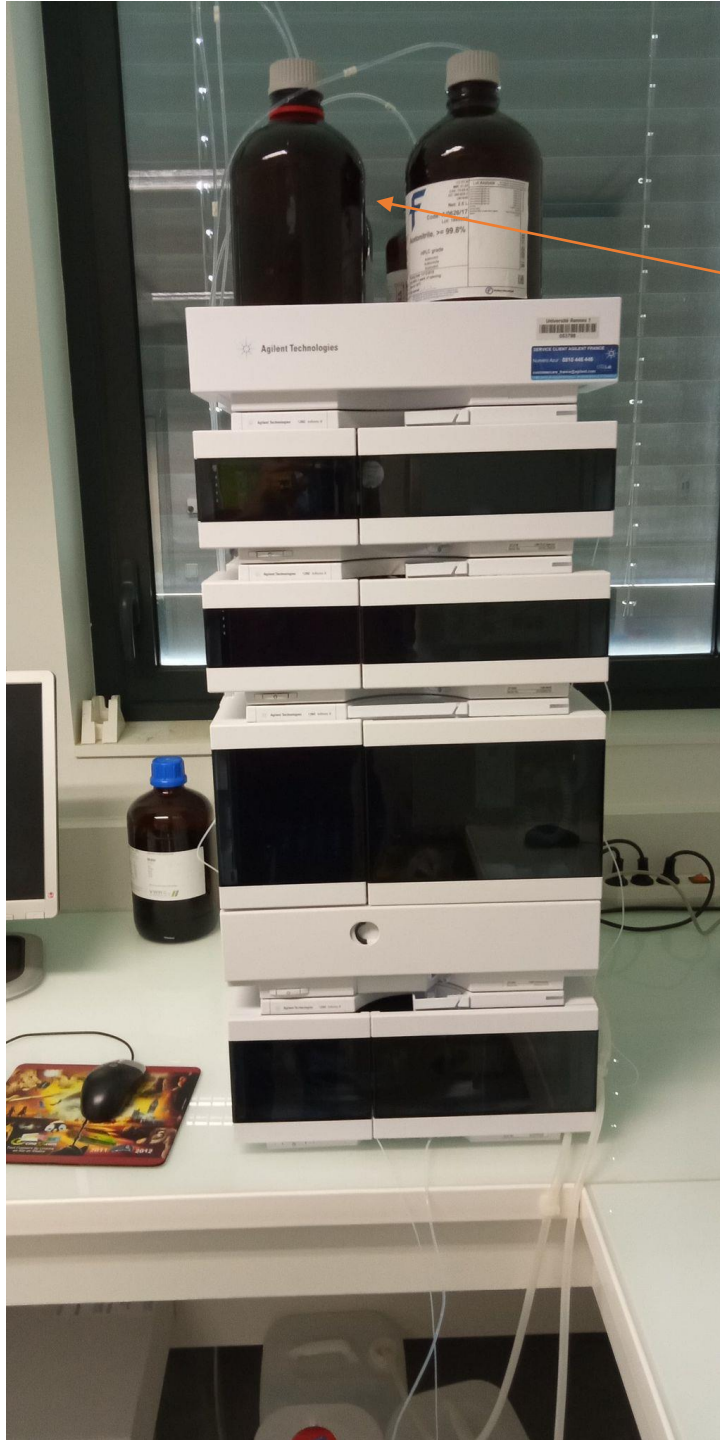


Appareil du bâtiment 7 à Villejean



Appareil du bâtiment 7 à Villejean

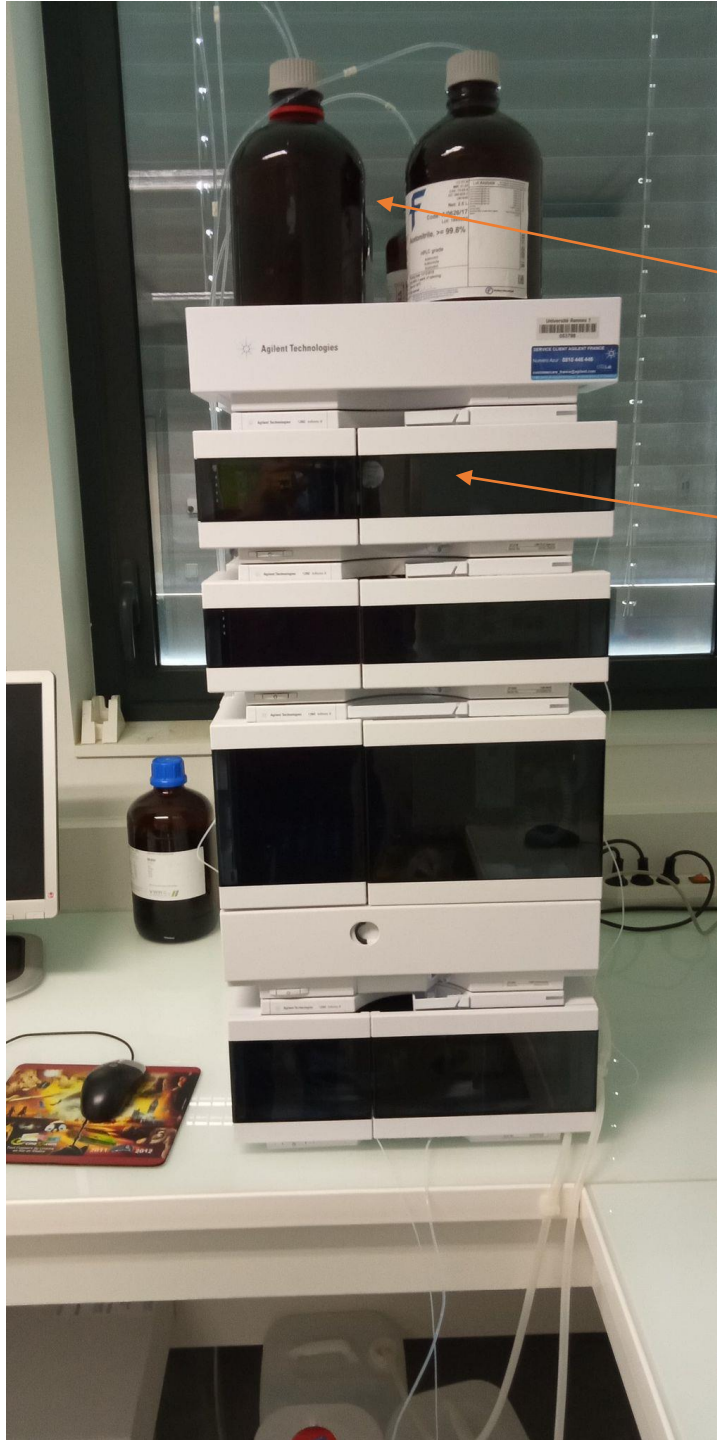
Solvants



Appareil du bâtiment 7 à Villejean

Solvants

Lecteur Fluorescence

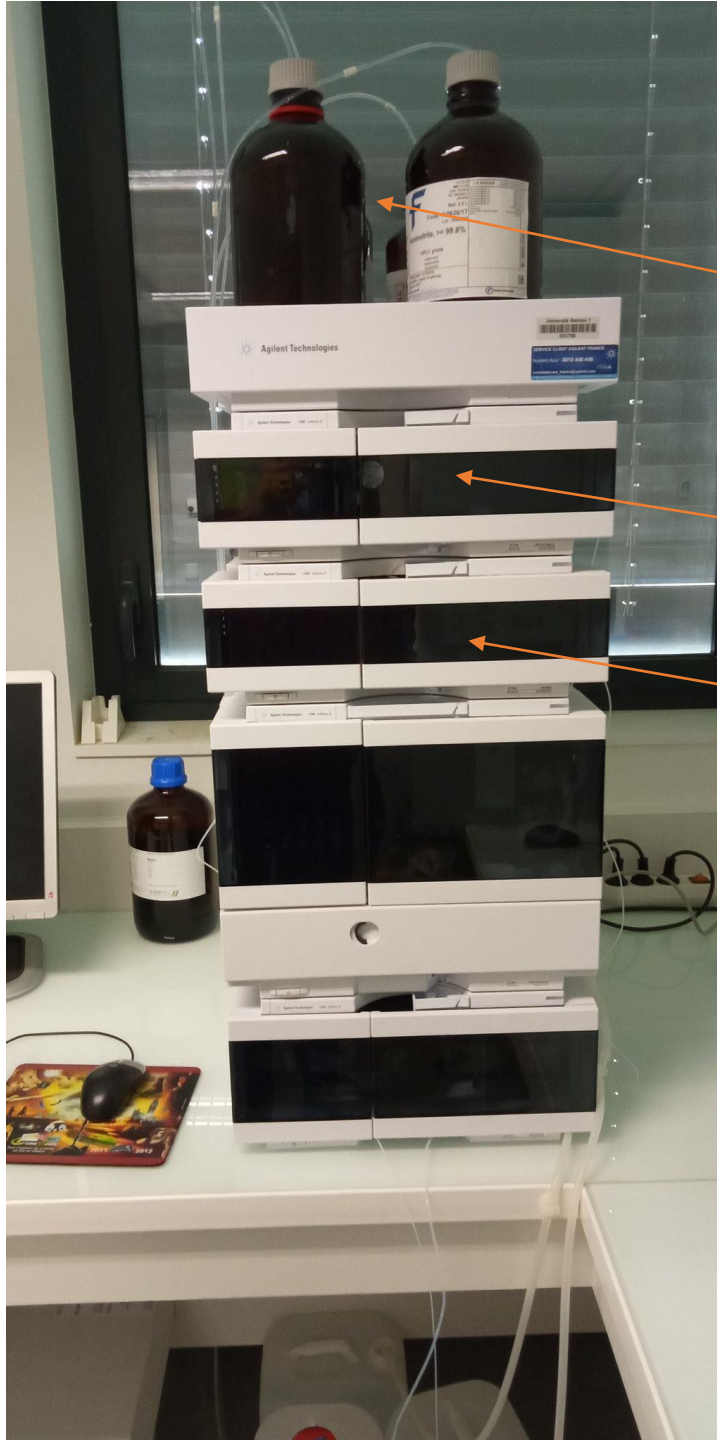


Appareil du bâtiment 7 à Villejean

Solvants

Lecteur Fluorescence

Lecteur UV



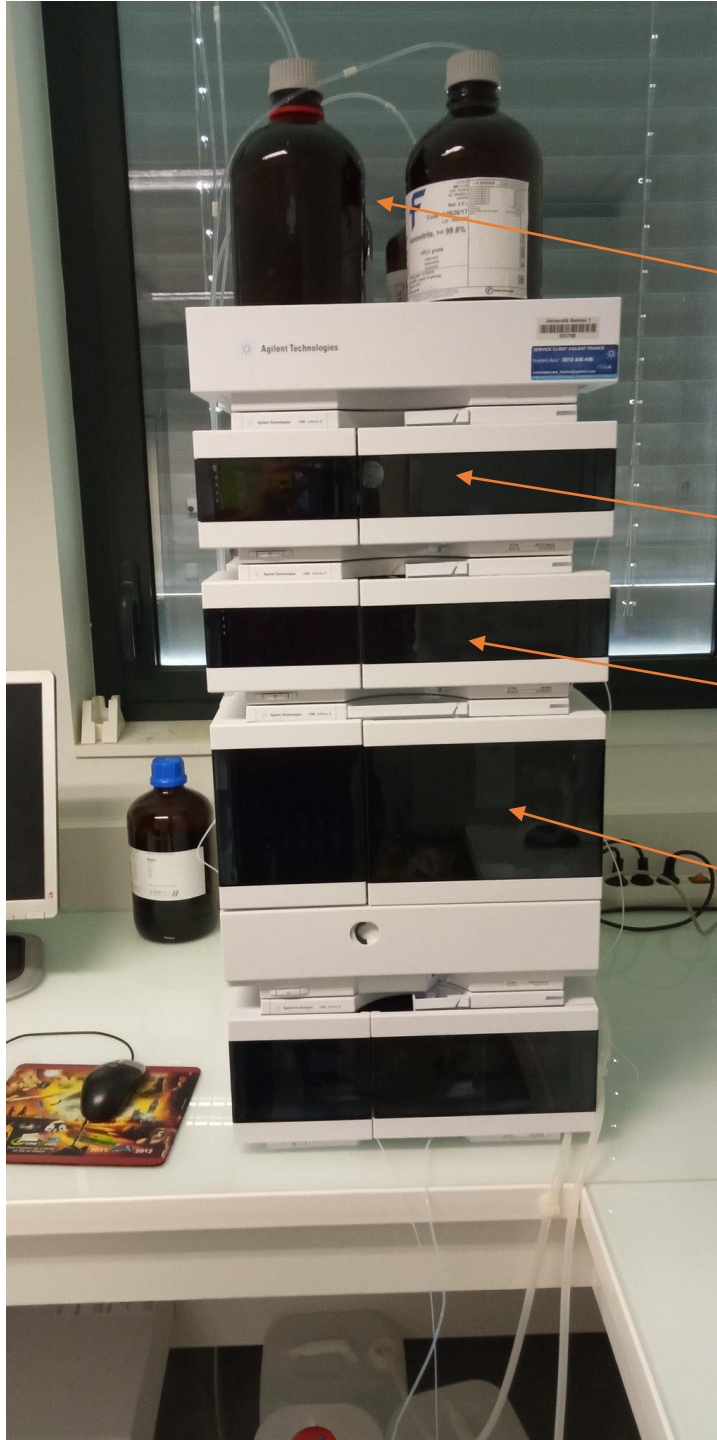
Appareil du bâtiment 7 à Villejean

Solvants

Lecteur Fluorescence

Lecteur UV

Echantillons +
bras Injecteur



Appareil du bâtiment 7 à Villejean

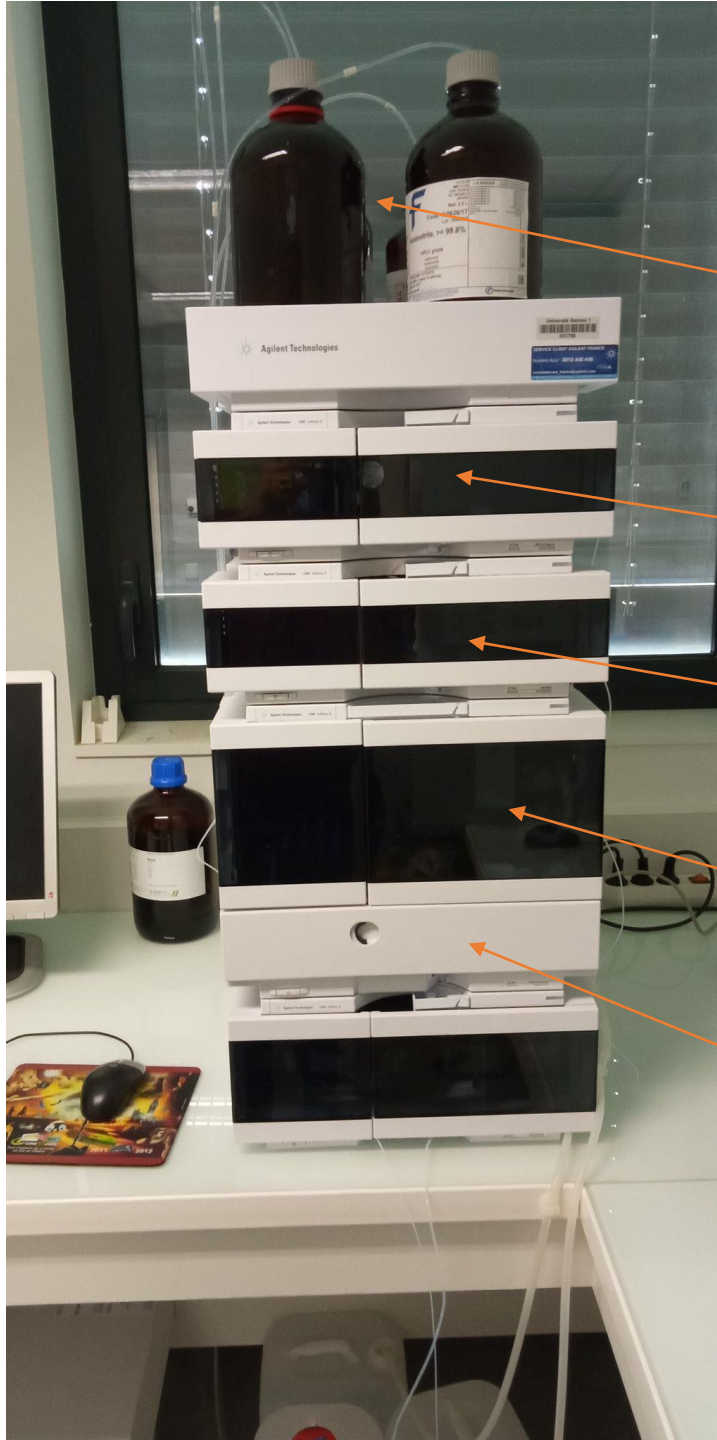
Solvants

Lecteur Fluorescence

Lecteur UV

Echantillons +
bras Injecteur

Four + colonne



Appareil du bâtiment 7 à Villejean

Solvants

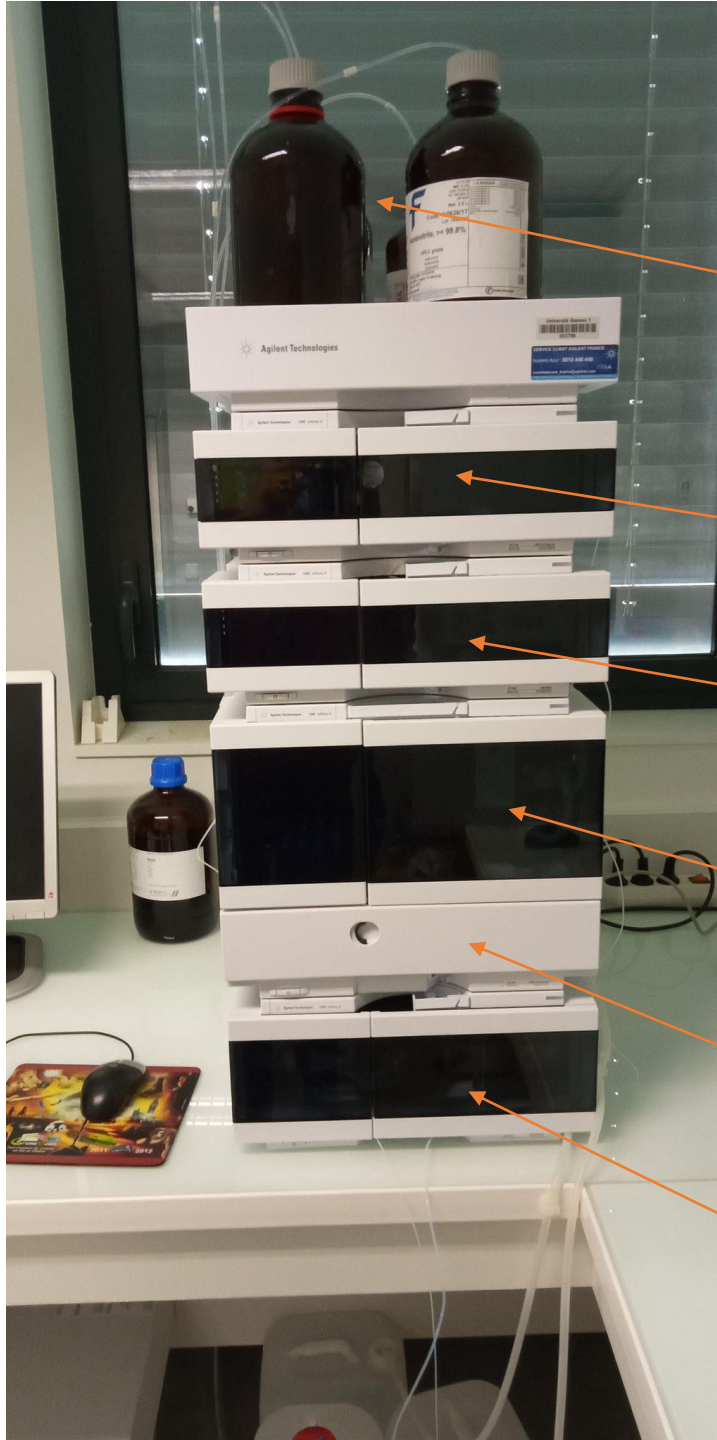
Lecteur Fluorescence

Lecteur UV

Echantillons +
bras Injecteur

Four + colonne

Pompe + purge



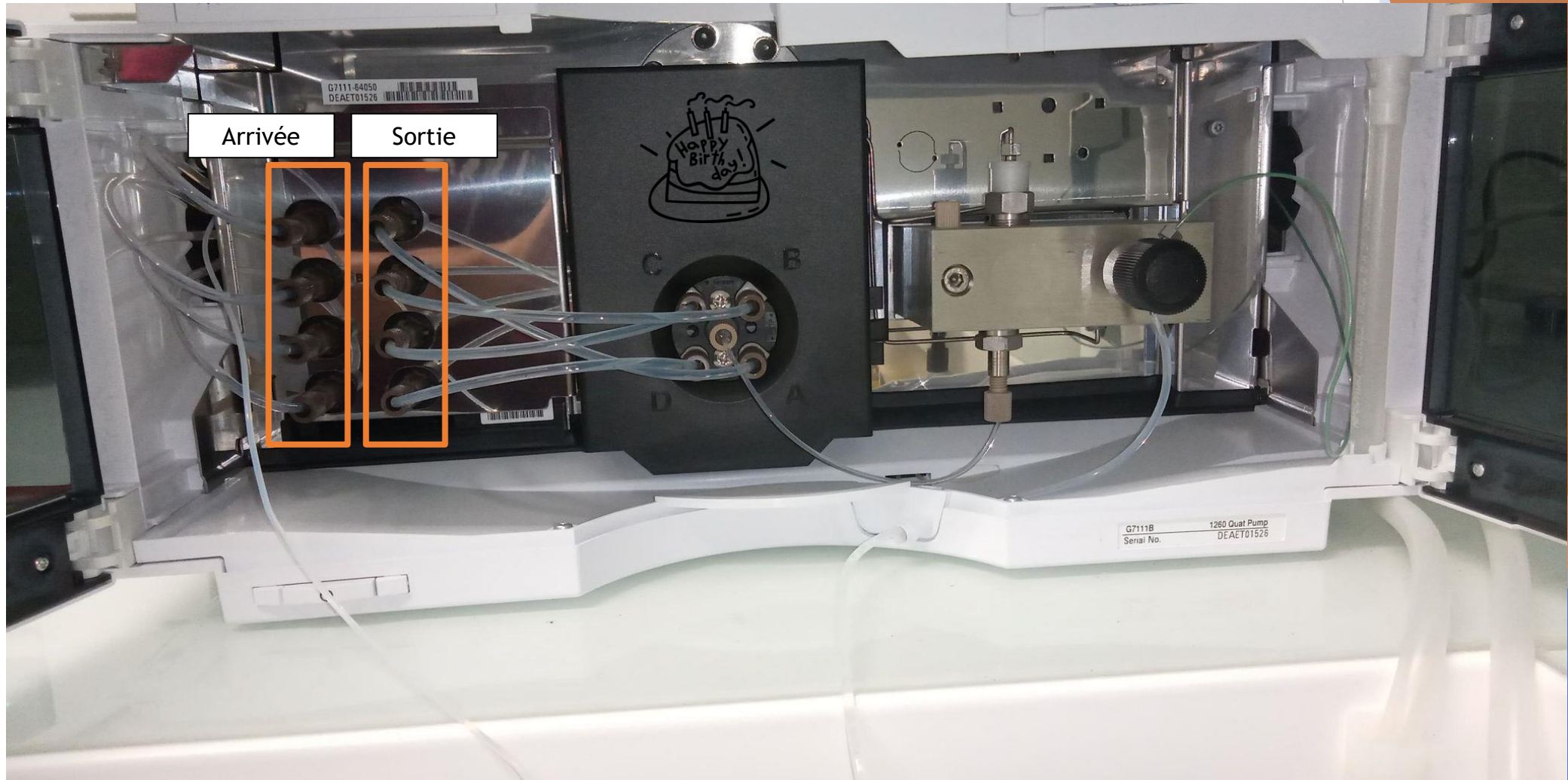
Pompe, mélangeur et purge



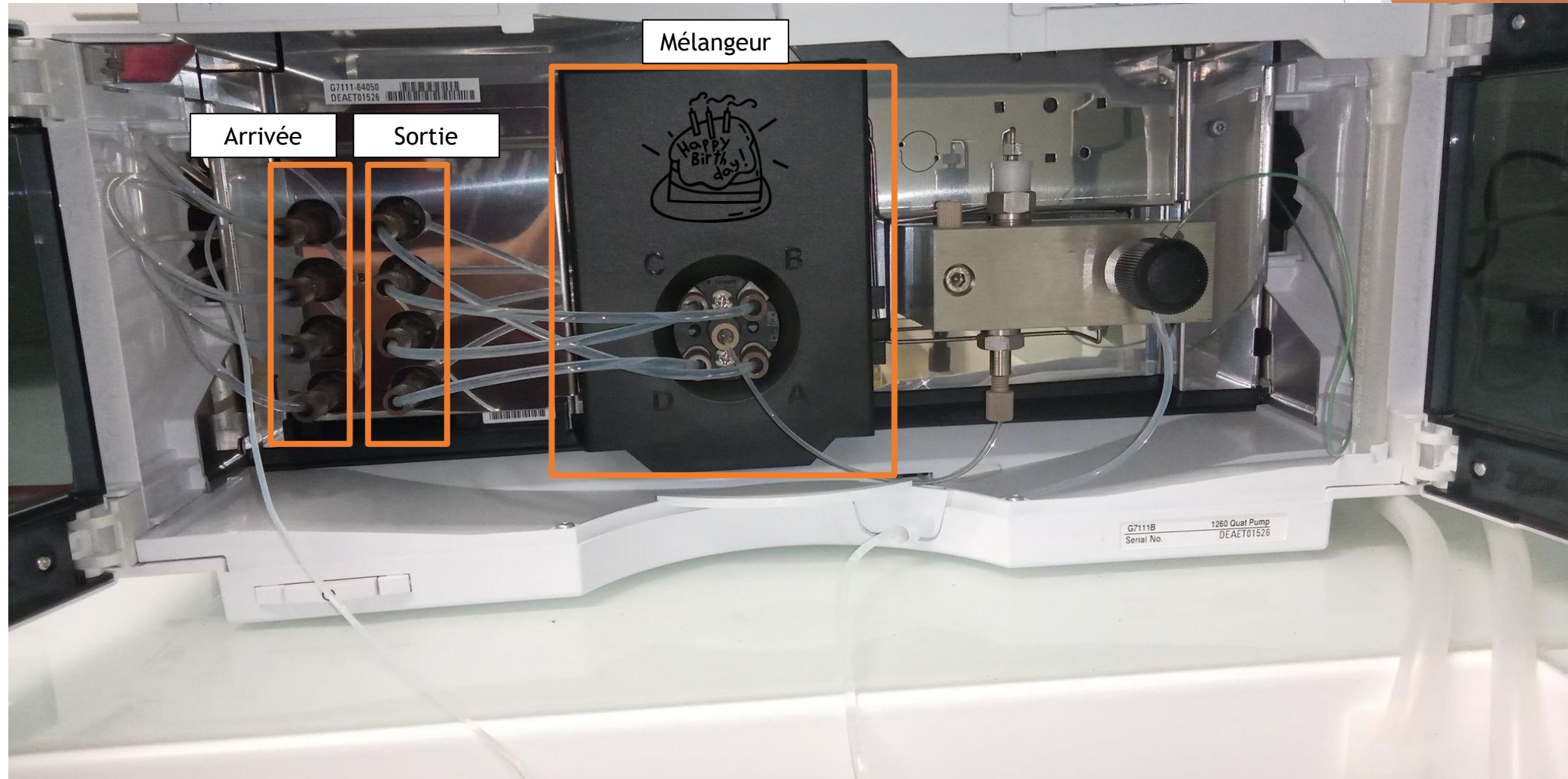
Pompe, mélangeur et purge



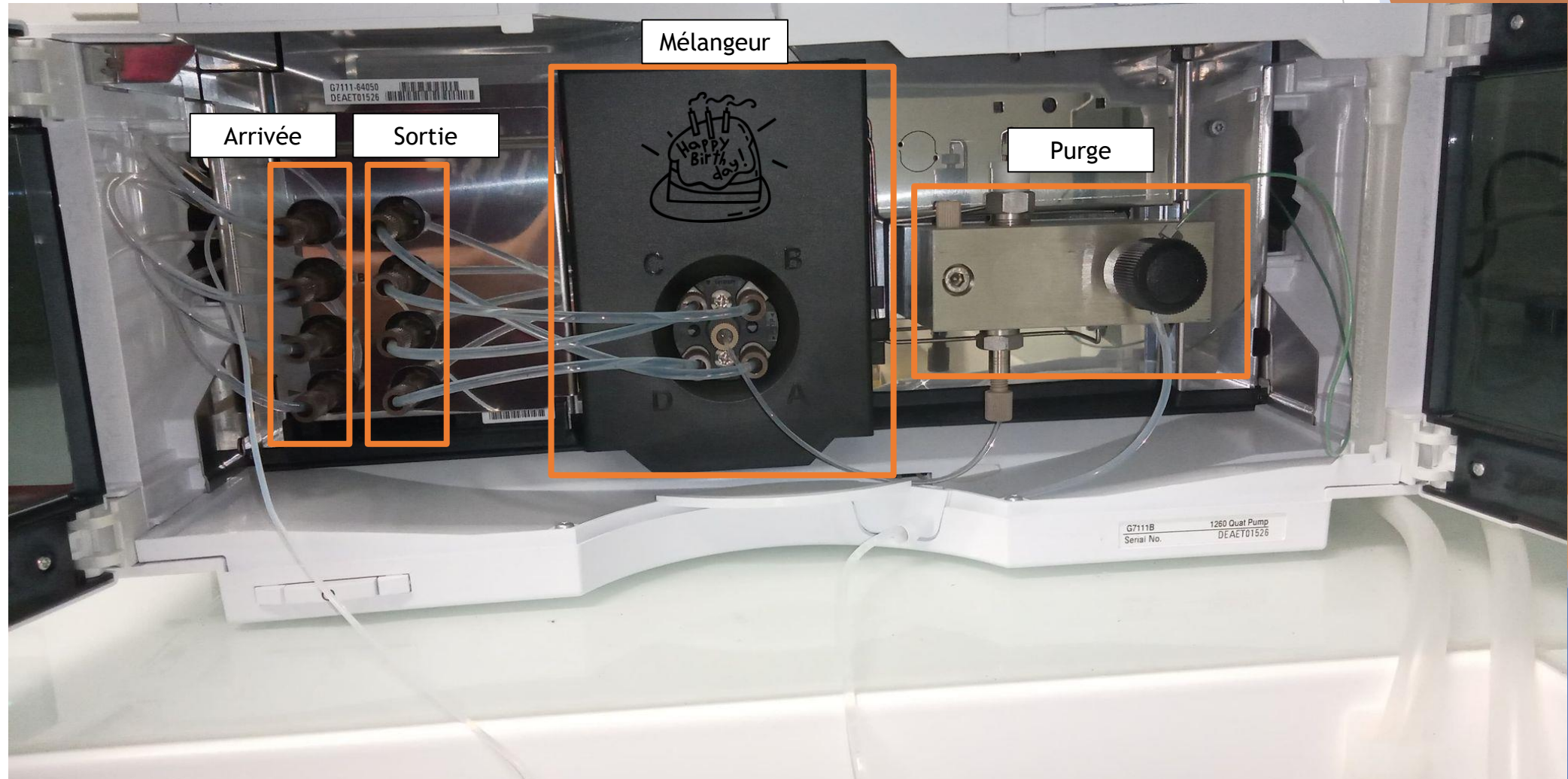
Pompe, mélangeur et purge



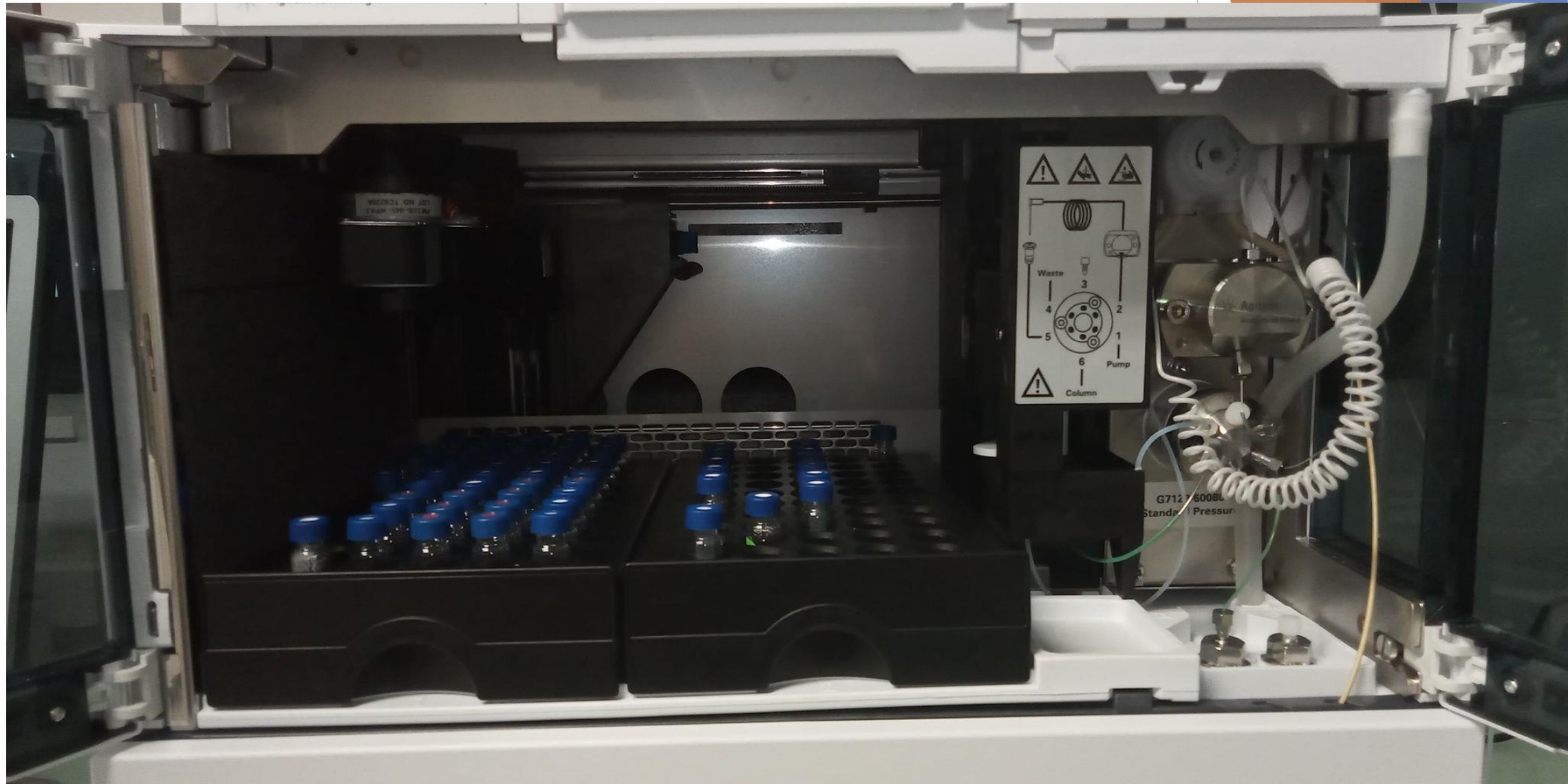
Pompe, mélangeur et purge



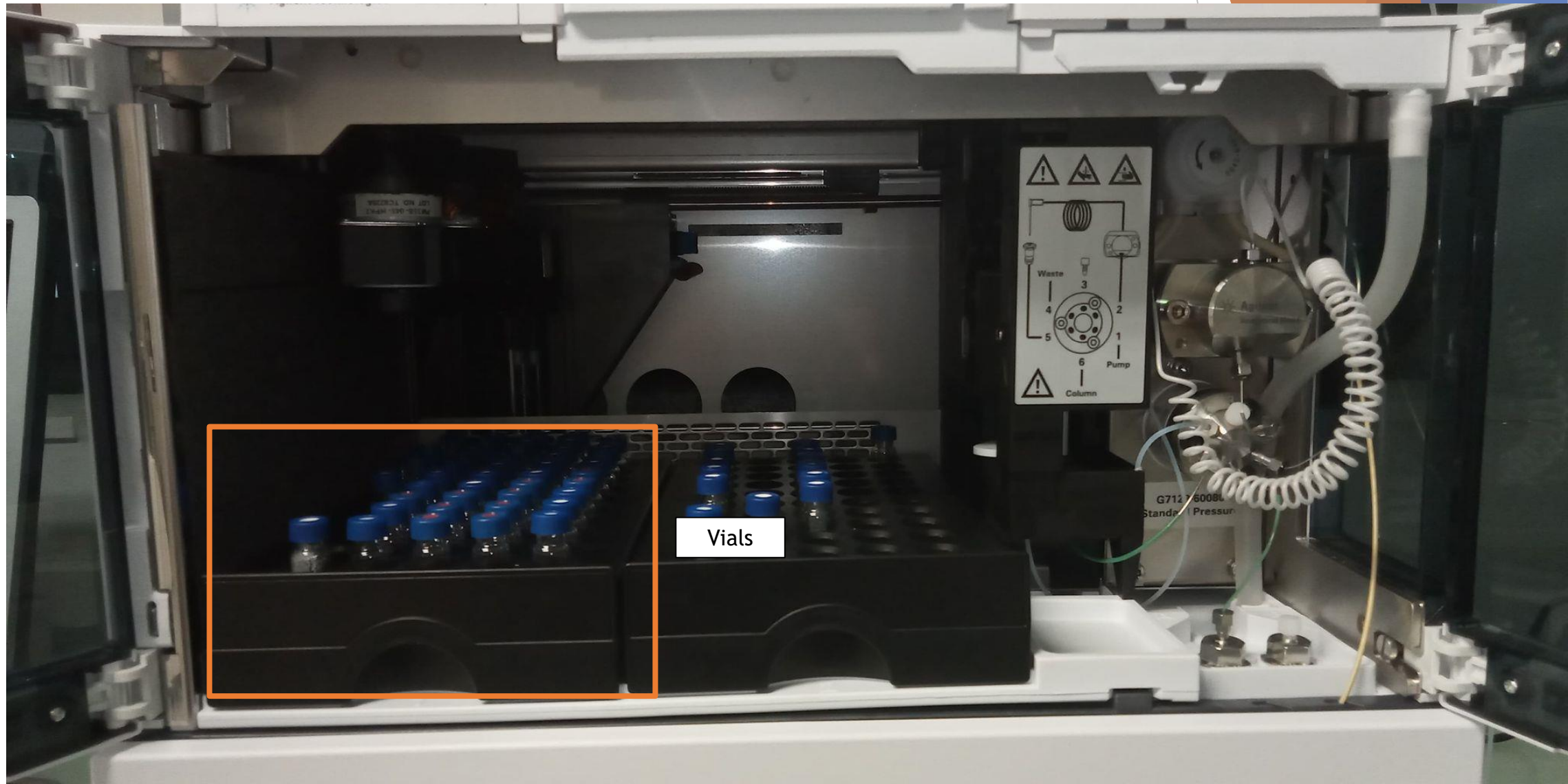
Pompe, mélangeur et purge



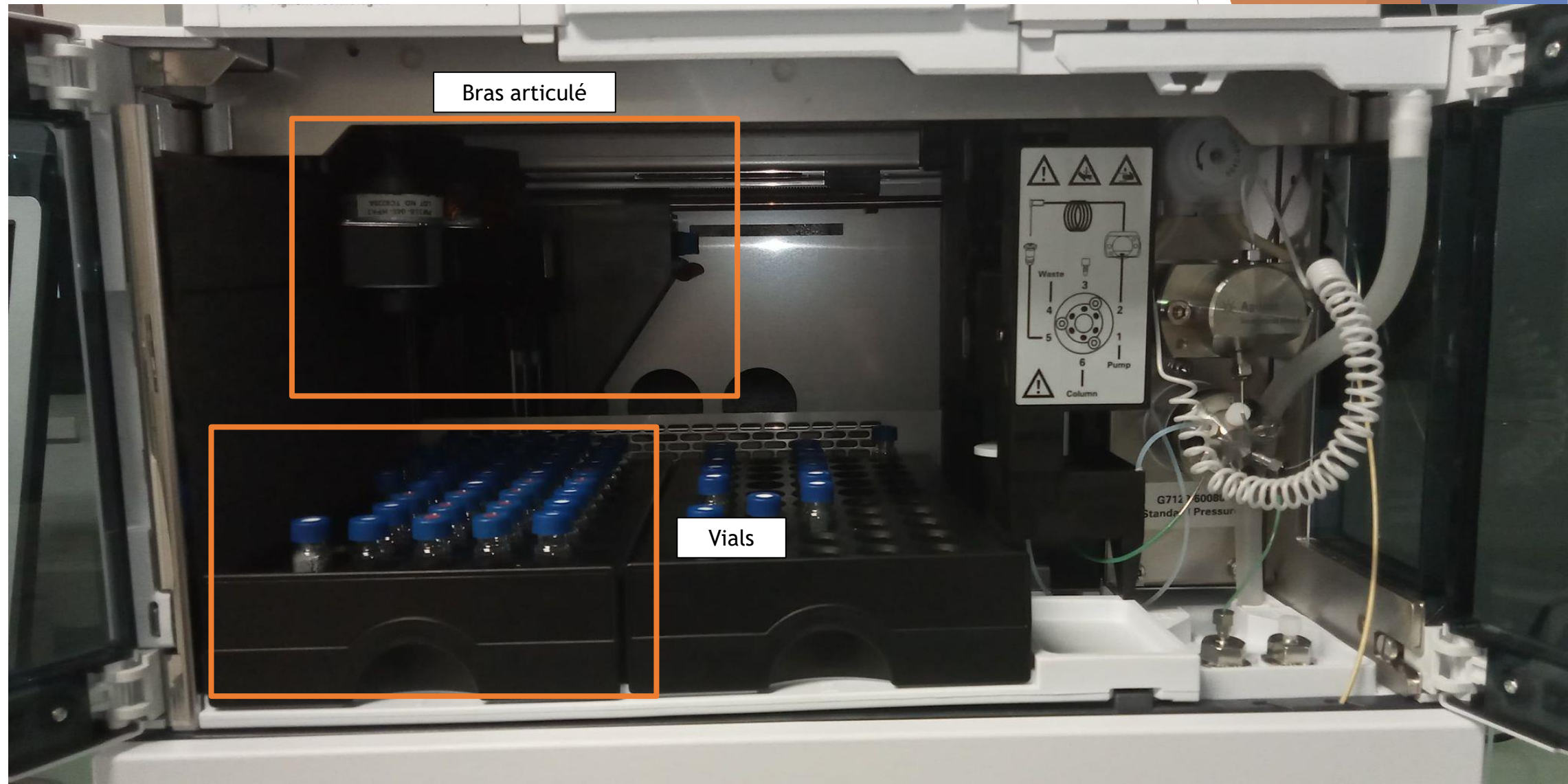
Rack d'échantillons et bras injecteur



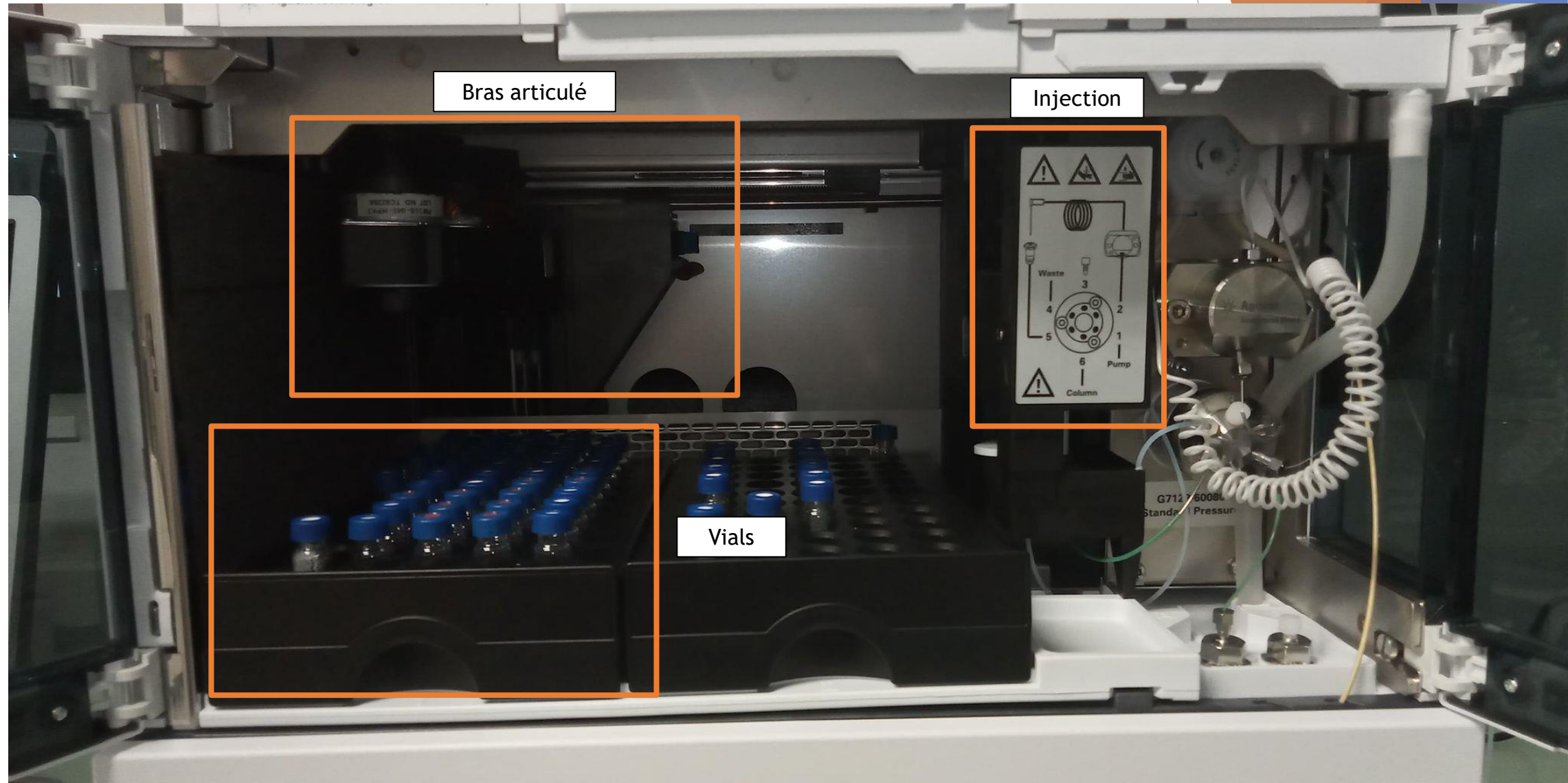
Rack d'échantillons et bras injecteur



Rack d'échantillons et bras injecteur



Rack d'échantillons et bras injecteur



Colonne et four



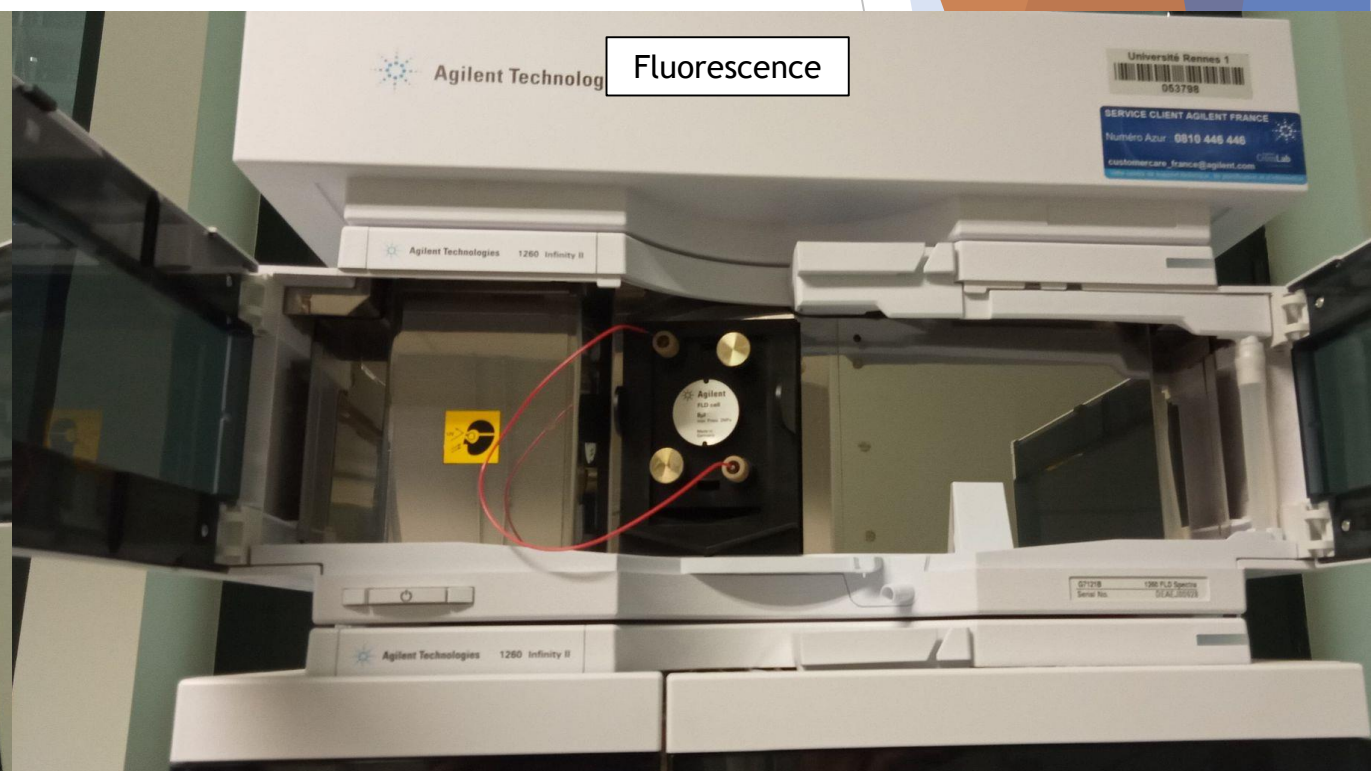
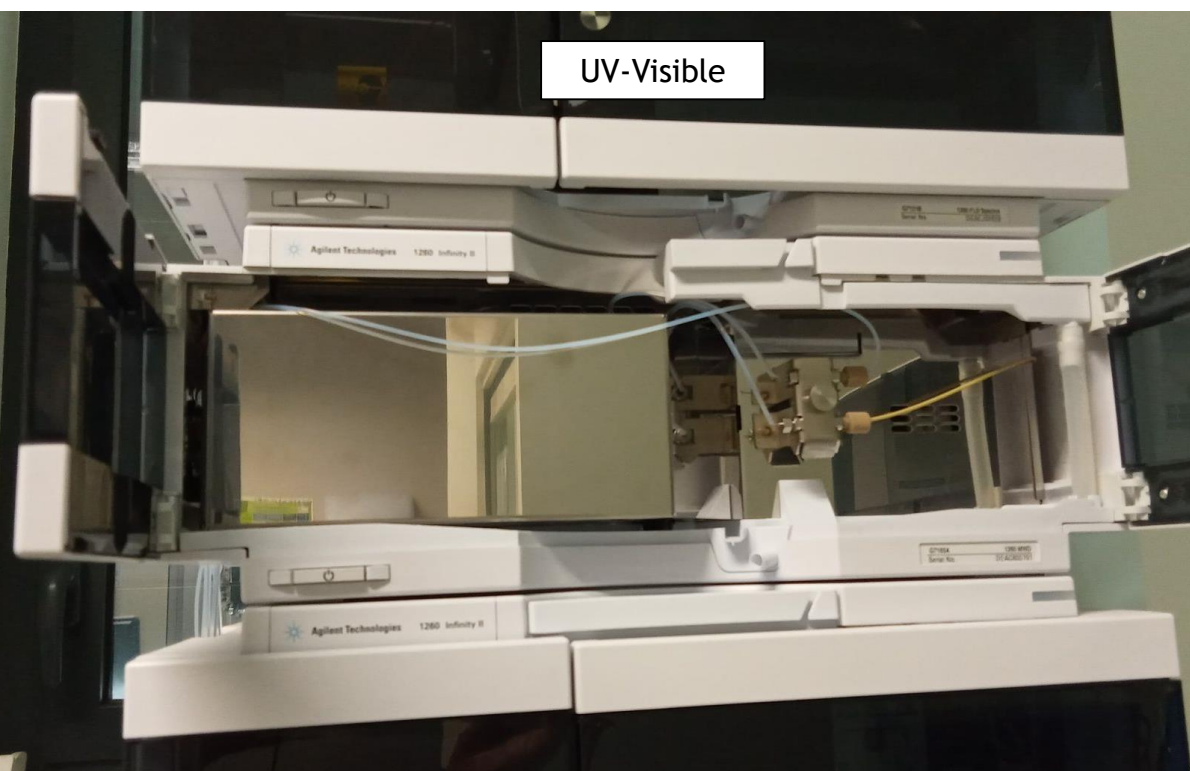
Colonne et four



Colonne et four



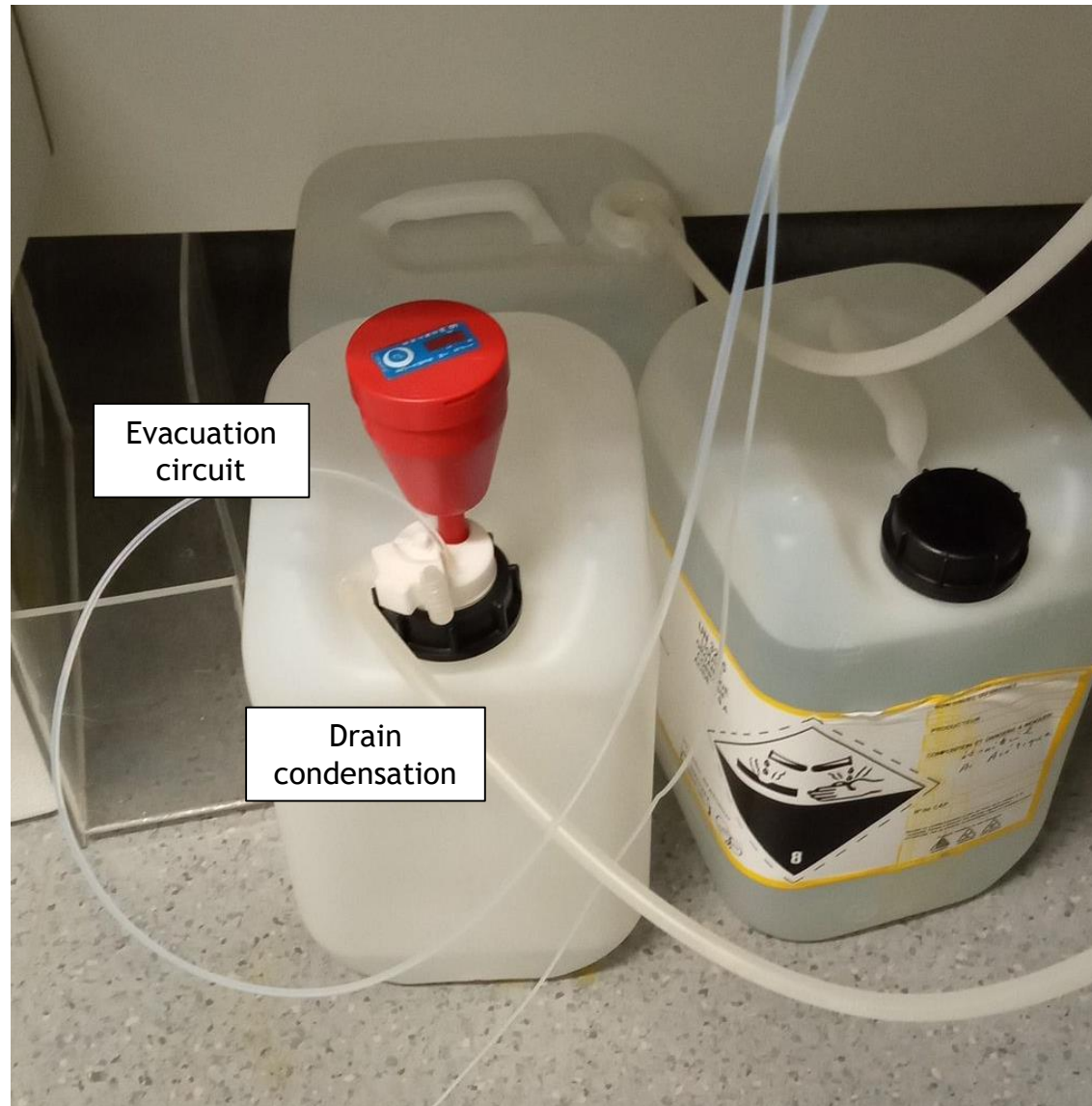
Lecteurs optiques (Ultraviolet et Fluorescence)



Poubelle



Poubelle



Interface « Online » du logiciel HPLC

Liste des protocoles

The screenshot displays the HPLC 1260 infinity II software interface. The 'Method and Run Control' window is open, showing the 'Methods' list on the left, which is highlighted with an orange box. The main panel displays the status of various instrument components: Sampler (Idle), Quat. Pump (Not Ready), Column Oven (Idle), MWD (Idle), and FLD (Idle). The Quat. Pump status is highlighted in yellow. The bottom section shows a table with sample information.

Location	Sample Name	Sample Info	Datafile	Data Dir.
7			7 2020-10-19 18-29-32.D	C:\data\

Interface « Online » du logiciel HPLC

Onglets d'analyse

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop PENICA.M1RINCAGE.M

Sampler EMF Idle 50.00 µL 27 °C

Quat. Pump EMF Not Ready 0.0 0.0 0.000 mL/min 5.22 bar 0.0 100.0

Column Oven EMF Idle 24.80 °C

MWD EMF Idle

FLD EMF Idle

0.00 / 0.00

Instrument Not Ready i On Off

Single Sample

Location	Sample Name	Sample Info	Datafile	Data Dir.
	7		7 2020-10-19 18-29-32.D	C:\data\

Data Analysis

Review

Report Layout

Verification (OQ/PV)

C:\895 GB C:\data\

SYSTEM (localhost) HPLC 1260 infinity II 0 Ready

Interface « Online » du logiciel HPLC

Affichage de la lecture
en cours

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop PENICA.M1RINCAGE.M

Sampler EMF Idle 50.00 µL 27 °C

Quat. Pump EMF Not Ready 0.0 0.0 0.000 mL/min 5.22 bar 0.0 100.0

Column Oven EMF Idle 24.80 °C

MWD EMF Idle

FLD EMF Idle

0.00 / 0.00 Instrument Not Ready On Off

Single Sample

Location	Sample Name	Sample Info	Datafile	Data Dir.
7			7 2020-10-19 18-29-32.D	C:\data\

Methods Sequence templates

Method and Run Control

Data Analysis

Review

Report Layout

Verification (OQ/PV)

C:\895 GB C:\data\

SYSTEM (localhost) HPLC 1260 infinity II 0 Ready

Interface « Online » du logiciel HPLC

Contrôle de l'appareil,
création de protocole

The screenshot displays the HPLC 1260 infinity II software interface, titled "HPLC 1260 infinity II (online): Method and Run Control". The interface is divided into several sections:

- Top Bar:** Shows the current method "PENICA.M1RINCAGE.M" and sequence "CYP3A4 Gamme test 10_19_2020.S". It includes a "Not Ready" status indicator and a "Last Run" button.
- Left Panel:** A tree view of methods and sequences, including "C:\Chem32\1\Methods" and "C:\Chem32\1\Sequences".
- Main Control Area:** A large panel with a blue border, containing five sub-panels for instrument components:
 - Sampler:** Shows a syringe icon, a volume of 50.00 µL, and a temperature of 27 °C.
 - Quat. Pump:** Shows four bottles (A, B, C, D) with volumes 0.0, 0.0, 0.0, and 100.0 respectively, and a flow rate of 0.000 mL/min at 5.22 bar.
 - Column Oven:** Shows a thermometer icon and a temperature of 24.80 °C.
 - MWD:** Shows a light bulb icon and a status of "Idle".
 - FLD:** Shows a Xe icon and a status of "Idle".
- Bottom Panel:** A table for "Single Sample" with columns for Location, Sample Name, Sample Info, Datafile, and Data Dir. The table shows a single entry with Sample Name "7" and Datafile "7 2020-10-19 18-29-32.D".
- Right Panel:** A "Data Analysis" section with icons for a chromatogram, a balance scale, and a data file.
- Bottom Bar:** A status bar showing "Instrument Not Ready" and buttons for "On" and "Off".

Interface « Online » du logiciel HPLC

Echantillons et
injecteur

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop PENICA.M1RINCAGE.M

Sampler EMF Idle 50.00 µL 27 °C 0.00 / 0.00

Quat. Pump EMF Not Ready 0.0 0.0 0.000 mL/min 0.0 100.0 5.22 bar

Column Oven EMF Idle 24.80 °C

MWD EMF Idle

FLD EMF Idle

Instrument Not Ready i On Off

Location	Sample Name	Sample Info	Datafile	Data Dir.
7			7 2020-10-19 18-29-32.D	C:\data\

Method and Run Control

Data Analysis

Review

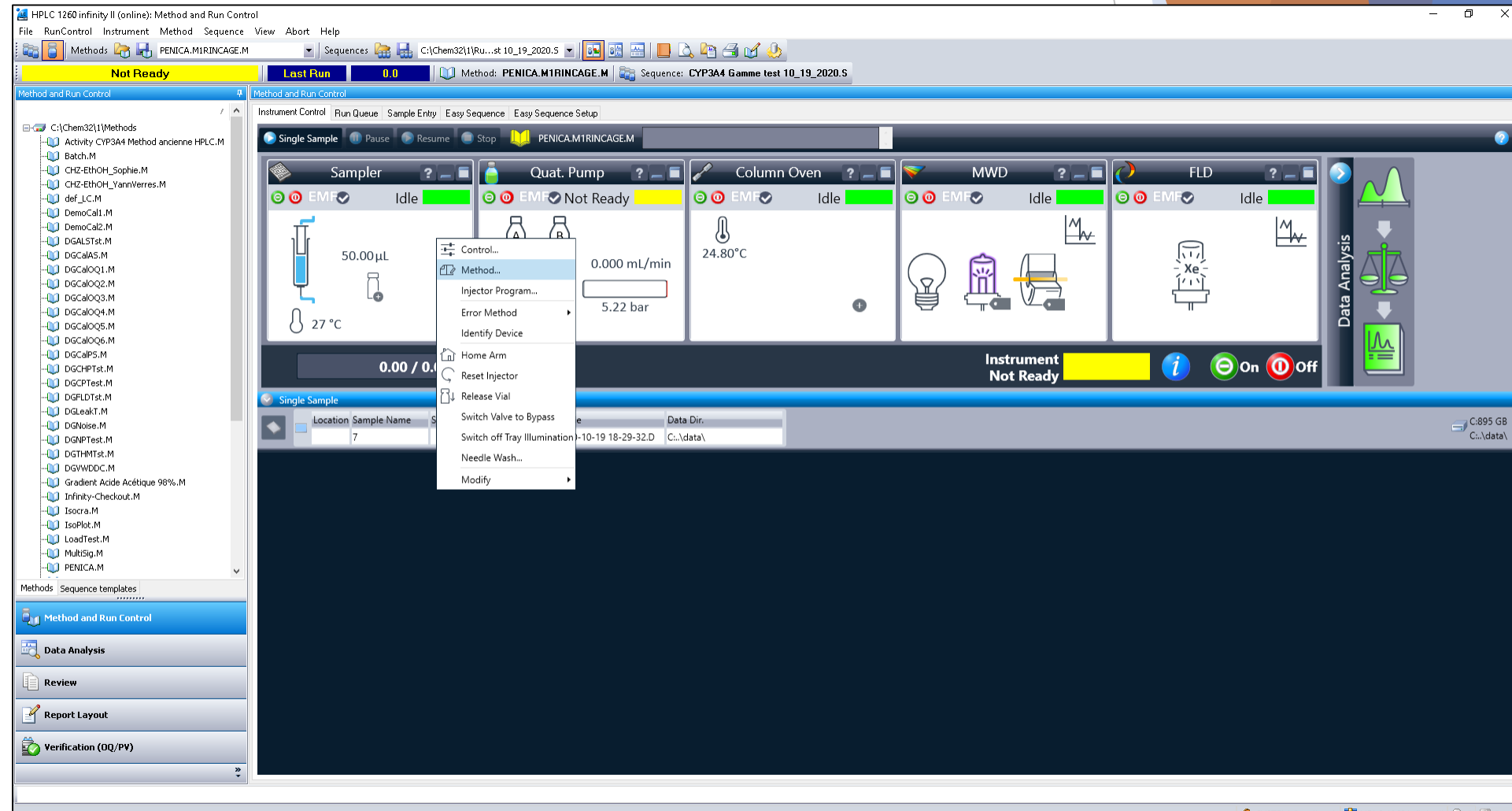
Report Layout

Verification (OQ/PV)

SYSTEM (localhost) HPLC 1260 infinity II 0 Ready

Interface « Online » du logiciel HPLC

Echantillons et
injecteur



Interface « Online » du logiciel HPLC

Echantillons et injecteur

-Volume d'injection

-Stoptime

The screenshot displays the HPLC 1260 infinity II software interface. The main window is titled "Method and Run Control" and shows a list of methods on the left, including "Activity CYP3A4 Method ancienne HPLC.M", "Batch.M", "CHZ-EthOH_Sophie.M", "CHZ-EthOH_YannVerres.M", "def_L.C.M", "DemoCal1.M", "DemoCal2.M", "DGCALSTst.M", "DGCALAS.M", "DGCALOQ1.M", "DGCALOQ2.M", "DGCALOQ3.M", "DGCALOQ4.M", "DGCALOQ5.M", "DGCALOQ6.M", "DGCALPS.M", "DGCPTst.M", "DGCPTst.M", "DGLFLDSt.M", "DGLLeakT.M", "DGLNoise.M", "DGNPTst.M", "DGTMTst.M", "DGTWDDC.M", "Gradient Acide Acétique 98%.M", "Infinity-Checkout.M", "Isocra.M", "IsotPlot.M", "LoadTest.M", "MultiSig.M", and "PENICA.M". The "Method and Run Control" window is open, showing the "Injection" section with "Injection volume: 50.00 µL" and "Needle wash" section with "Enable Needle Wash" checked. The "Stoptime" section shows "As Pump/No Limit" selected. The "Sampler (G7129A)" configuration dialog is open, showing the "Advanced" section with "Auxiliary" settings: "Draw speed: 200 µL/min", "Eject speed: 400 µL/min", "Wait Time After Draw: 1.2 s", and "Needle Height Offset: 0.0 mm". The "High throughput" section shows "Injection Valve to Bypass for Delay Volume Reduction" unchecked, "Sample Flush-Out Factor: 5.0 times injection volume", and "Overlapped Injection Mode: Off". The "Data Analysis" section on the right shows a chromatogram and a balance icon. The status bar at the bottom indicates "SYSTEM (localhost)" and "HPLC 1260 infinity II".

Interface « Online » du logiciel HPLC

Solvants et pompe

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop PENICA.M1RINCAGE.M

Sampler Quat. Pump Column Oven MWD FLD

EMF Idle EMF Not Ready EMF Idle EMF Idle EMF Idle

50.00 µL 27 °C 0.0 0.0 0.000 mL/min 24.80 °C

0.0 100.0 5.22 bar

0.00 / 0.00 Instrument Not Ready

Single Sample

Location	Sample Name	Sample Info	Datafile	Data Dir.
	7		7 2020-10-19 18-29-32.D	C:\data\

Method and Run Control

Data Analysis

Review

Report Layout

Verification (OQ/PV)

SYSTEM (localhost) HPLC 1260 infinity II 0 Ready

Interface « Online » du logiciel HPLC

Solvants et pompe

- Solvants utilisés
- % des solvants
- Temps de chaque phase
- Débit
- Pression max
- Stoptime

The screenshot displays the HPLC 1260 infinity II software interface. The main window is titled "Method and Run Control" and shows the "Method of G7111B (DEAET01526)". The "Flow" section indicates a flow rate of 0.500 mL/min. The "Solvents" section lists four solvents: A (0.0% Acetonitrile), B (0.0% H2O), C (0.0% H2O), and D (100.0% Ac. Acétique 0.1%). The "Pressure Limits" section shows a minimum pressure of 0.00 bar and a maximum pressure of 600.00 bar. The "Stoptime" section shows a stop time of 10.00 min. The "Advanced" section displays a timetable with the following data:

Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	0.0	0.0	0.0	100.0	0.500	600.00
1.00	0.0	90.0	10.0	0.0	0.500	600.00
10.00	0.0	90.0	10.0	0.0	0.500	600.00

The interface also includes a "Data Analysis" section on the right, showing a chromatogram and a balance icon. The status bar at the bottom indicates the system is ready and the data is stored in C:\data\.

Interface « Online » du logiciel HPLC

Colonne et four

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop PENICA.M1RINCAGE.M

Sampler Quat. Pump Column Oven MWD FLD

EMF Idle 50.00 µL 27 °C

EMF Not Ready 0.0 0.0 0.000 mL/min 5.22 bar

EMF Idle 24.80 °C

EMF Idle

EMF Idle

0.00 / 0.00

Instrument Not Ready

Single Sample

Location	Sample Name	Sample Info	Datafile	Data Dir.
	7		7 2020-10-19 18-29-32.D	C:\data\

Method and Run Control

Data Analysis

Review

Report Layout

Verification (OQ/PV)

SYSTEM (localhost) HPLC 1260 infinity II

Interface « Online » du logiciel HPLC

Capteur UV-Visible

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop PENICA.M1RINCAGE.M

Sampler Quat. Pump Column Oven MWD FLD

EMF Idle 50.00 µL 27 °C

EMF Not Ready 0.0 0.0 0.000 mL/min 5.22 bar

EMF Idle 24.80 °C

EMF Idle

EMF Idle

0.00 / 0.00

Instrument Not Ready

Single Sample

Location	Sample Name	Sample Info	Datafile	Data Dir.
	7		7 2020-10-19 18-29-32.D	C:\data\

Method and Run Control

Data Analysis

Review

Report Layout

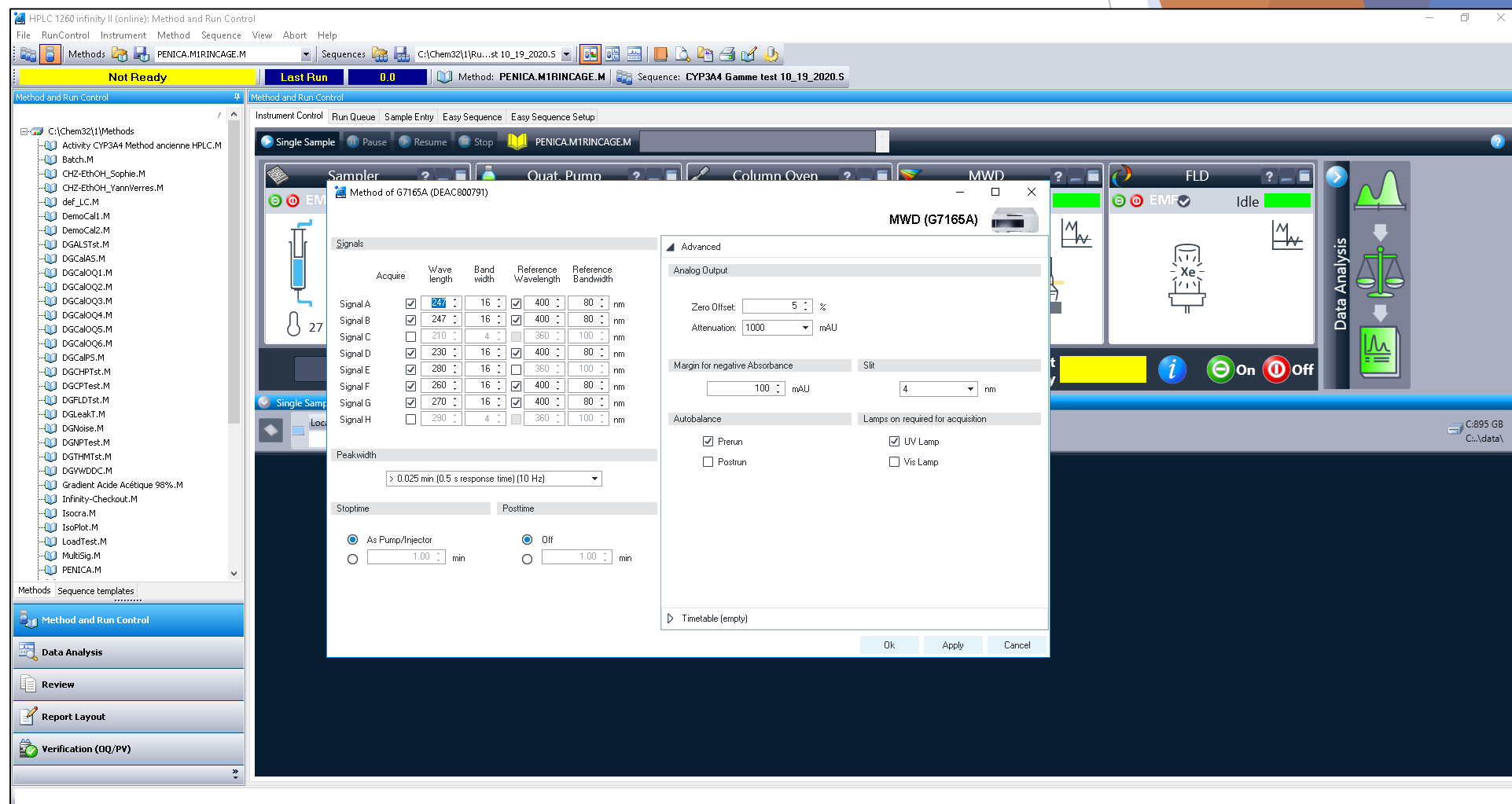
Verification (OQ/PV)

SYSTEM (localhost) HPLC 1260 infinity II

Interface « Online » du logiciel HPLC

Capteur UV-Visible

- Longueur d'onde de détection
- Largeur de la lecture autour de la valeur
- Longueur d'onde de référence



Interface « Online » du logiciel HPLC

Capteur Fluorescence

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop PENICA.M1RINCAGE.M

Sampler Quat. Pump Column Oven MWD FLD

EMF Idle 50.00 µL 27 °C

EMF Not Ready 0.0 0.0 0.000 mL/min 5.22 bar

EMF Idle 24.80 °C

EMF Idle

EMF Idle

0.00 / 0.00

Instrument Not Ready

On Off

Single Sample

Location	Sample Name	Sample Info	Datafile	Data Dir.
	7		7 2020-10-19 18-29-32.D	C:\data\

Method and Run Control

Data Analysis

Review

Report Layout

Verification (OQ/PV)

SYSTEM (localhost) HPLC 1260 infinity II

Interface « Online » du logiciel HPLC

The screenshot displays the HPLC 1260 infinity II software interface, specifically the 'Method and Run Control' window. The window is divided into several sections:

- File Explorer:** Located on the left, it shows a tree view of files and folders. The 'Methods' folder is expanded, showing a list of methods including 'Activity CYP3A4 Method', 'Batch.M', 'CHZ-EthOH_Sophie.M', 'CHZ-EthOH_YannVerres', 'def_LC.M', 'DemoCal1.M', 'DemoCal2.M', 'DGALST.M', 'DGCALAS.M', 'DGCALOQ1.M', 'DGCALOQ2.M', 'DGCALOQ3.M', 'DGCALOQ4.M', 'DGCALOQ5.M', 'DGCALOQ6.M', 'DGCALPS.M', 'DGCHPTst.M', 'DGCPTst.M', 'DGFLDTst.M', 'DGLeakT.M', 'DGNoise.M', 'DGNPTst.M', 'DGTHTst.M', 'DGVWDC.M', 'Gradient Acide Acétique 98%.M', 'Infinity-Checkout.M', 'Isocra.M', 'IsoPlot.M', 'LoadTest.M', 'MultiSig.M', and 'PENICA.M'.
- Method and Run Control:** The main area of the window, showing the current method 'PENICA.M1RINCAGE.M' and sequence 'CYP3A4 Gamme test 10_19_2020.S'. It includes a 'Resume' button and a 'Stop' button.
- Instrument Status Panels:** Four panels at the top right show the status of different components: 'Quat. Pump' (Not Ready), 'Column Oven' (Idle), 'MWD' (Idle), and 'FLD' (Idle). Each panel includes a temperature reading and a status indicator.
- Data Analysis:** A vertical panel on the right side of the window, featuring a green balance scale icon and a green bar chart icon.
- Sample Table:** A table at the bottom left showing sample information. The table has columns for 'Location', 'Sample Name', 'Sample Info', 'Datafile', and 'Data Dir.'. The first row shows '7' in the 'Sample Name' column and '7 2020-10-19 18-29-32.D' in the 'Datafile' column.
- Bottom Bar:** A blue bar at the bottom of the window with buttons for 'Method and Run Control', 'Data Analysis', 'Review', 'Report Layout', and 'Verification (OQ/PV)'. It also includes a status indicator 'Instrument Not Ready' and a 'Data Analysis' button.

The status bar at the bottom of the window shows 'SYSTEM (localhost)' and 'HPLC 1260 infinity II'.

Interface « Online » du logiciel HPLC

Séquence d'échantillons

- Emplacement de l'échantillon
- Nom/Identifiant
- Protocole appliqué

HPLC 1260 infinity II (online): Method and Run Control

File RunControl Instrument Method Sequence View Abort Help

Methods PENICA.M1RINCAGE.M Sequences C:\Chem32\1\Ru...st 10_19_2020.5 Method: PENICA.M1RINCAGE.M Sequence: CYP3A4 Gamme test 10_19_2020.S

Not Ready Last Run 0.0

Method and Run Control

Instrument Control Run Queue Sample Entry Easy Sequence Easy Sequence Setup

Sampler - Half Trays Classic - Classic

Container

50VialsLeft

Sample List - CYP3A4 Gamme test 10_19_2020

#	Sample Location	Sample Name	Sample Info	Method Name	Inj Volume	Sample Type	Cal Level	Update RF	Update RT	Sample Amount	Multiplier	Dilution	ISTD1 Amount	ISTD1
1	1	H2O		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
2	2	60H Testosterone 100µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
3	3	60H Testosterone 50µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
4	4	60H Testosterone 25µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
5	5	60H Testosterone 12.5µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
6	6	60H Testosterone 6.25µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
7	7	60H Testosterone 3.125µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
8	8	60H Testosterone 1.562µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
9	9	60H Testosterone 0.781µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
10	10	60H Testosterone 0 µM		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
11	11	H-158		Activity CYP3A4...		Sample		Average	Average	0	1	1	0	
12	12	H-164		Activity CY...		Sample		Average	Average	0	1	1	0	
13	1	H2O		PENICA.M1rincage		Sample		Average	Average	0	1	1	0	

Total number of samples: 13 Add to queue

SYSTEM (localhost) HPLC 1260 infinity II

Avant chaque run



Connect

NuMeCan - Agilent Lab Advisor

Agilent Lab Advisor System Overview Basic Version

Tasks

- Lab Advisor
 - System Overview
 - Configuration
 - Apps
 - Firmware Update
 - Logs & Results
- NuMeCan
 - Service & Diagnostics
 - Instrument Control
 - EMF
 - System Report
- Help
 - Context Help (F1)
 - Help Topics
 - What's New?

System Overview

System Name	System Information	EMF	Status
NuMeCan	Agilent LC / 192.168.254.11	EMF?	Offline Connect
G7111B 1260 Quat Pump	Serial # DEAE101526 Firmware: D.07.24 [0001] - Degasser - LAN Settings (IP: 192.168.254.11, SM: 255.255.255.255)	EMF?	Connect
G7129A 1260 Vialmate	Serial # DEAEQ17313 Firmware: D.07.23 [0009] - Sample Thermostat (Product# G7167-60101, S... - Left Tray: 50 x 2 ml left, Right Tray: 50 x 2 ml right - Melting Parameter (Seal assembly PEEK 0.17 ... - LAN Settings (IP: 192.168.254.11, SM: 255.255.255.255)	EMF?	Connect
G7130A ICC	Serial # DEAEQ17313 Firmware: D.07.23 [0009] - Integral Type: G7129A - 6.0 uL Heat Exchanger - Internal Serial Number: DEBA804826	EMF?	Connect
G7121B 1260 FLD Spectra	Serial # DEAEJ00928 Firmware: D.07.24 [0001] - 8 µl Flow Cell - LAN Settings (IP: 192.168.254.11, SM: 255.255.255.255)	EMF?	Connect
G7165A 1260 MWD	Serial # DEAC800791 Firmware: D.07.23 [0009] - Flow Cell (Product# G1315-60022, Serial# DE1... - UV Lamp (Product# 2140-0520, Serial# 150021) - LAN Settings (IP: 192.168.254.11, SM: 255.255.255.255) - Current LAN Controller: 192.168.254.10 PC-C2...	EMF?	Connect

Fast Connect Remove System System Properties... Add System...

Connection Address: 192.168.254.11 Version B.02.10 [253] SP1 - Basic | Licenses 5/10 ...

Avant chaque run



Agilent Lab Advisor - System Overview

Tasks: Lab Advisor, System Overview, Configuration, Apps, Firmware Update, Logs & Results, NuMeCan, Service & Diagnostics, Instrument Control, EMFs, System Report

System Name	System Information	EMF?	Status
NuMeCan	Agilent LC / 192.168.254.11	EMF?	Offline
G7111B	1260 Quat Pump Serial # DEAE101526 Firmware: D.07.24 [0001]	EMF?	Offline
G7129A	1260 Vialsampler Serial # DEAEQ17313 Firmware: D.07.23 [0009]	EMF?	Offline
G7130A	ICC Serial # DEAEQ17313 Firmware: D.07.23 [0009]	EMF?	Offline
G7121B	1260 FLD Spectra Serial # DEAEJ00928 Firmware: D.07.24 [0001]	EMF?	Offline
DEAC800791	1260 MWD Serial # DEAC800791 Firmware: D.07.23 [0009]	EMF?	Offline

Fast Connect Remove System System Properties... Add System...

Connection Address: 192.168.254.11 Version B.02.10 [253] SP1 - Basic | Licenses 5/10

Agilent Lab Advisor - Service & Diagnostics

Tasks: Lab Advisor, System Overview, Configuration, Apps, Firmware Update, Logs & Results, NuMeCan, Service & Diagnostics, Instrument Control, EMFs, System Report

Filter: Tests, Calibrations, Tools

System Name: G7111B 1260 Quat Pump
Serial # DEAE101526

System Pressure Test
Diagnostic Buffers
Module Info
Purge Pump

Name: Pump Leak Rate Test
Approx. Time: 10 min
Description: The test determines the leak rates in the primary and the secondary pump chambers for component level diagnostic.

more... Run

Connection Address: 192.168.254.11 Version B.02.10 [253] SP1 - Basic | Licenses 5/10

Connect

Service & Diagnostic

Purge Pump

Avant chaque run

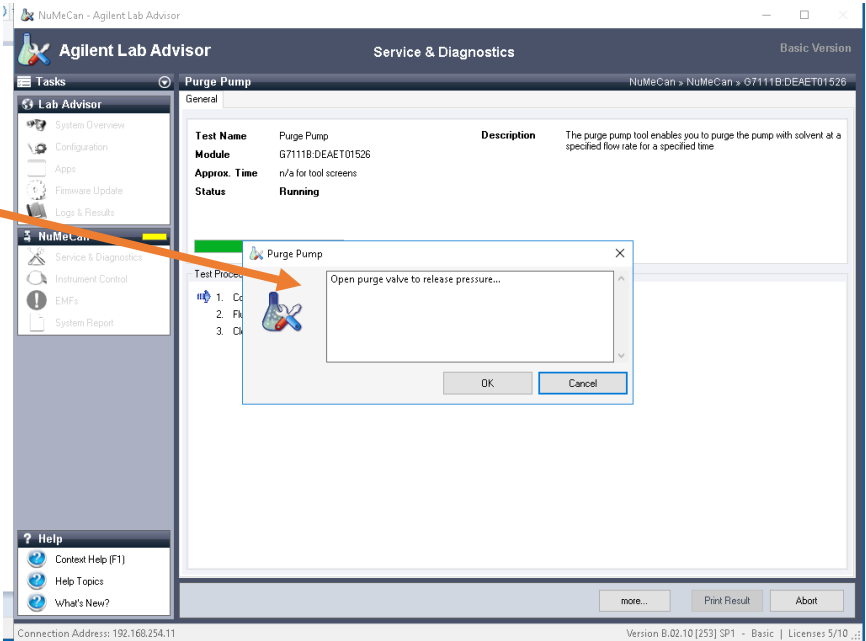
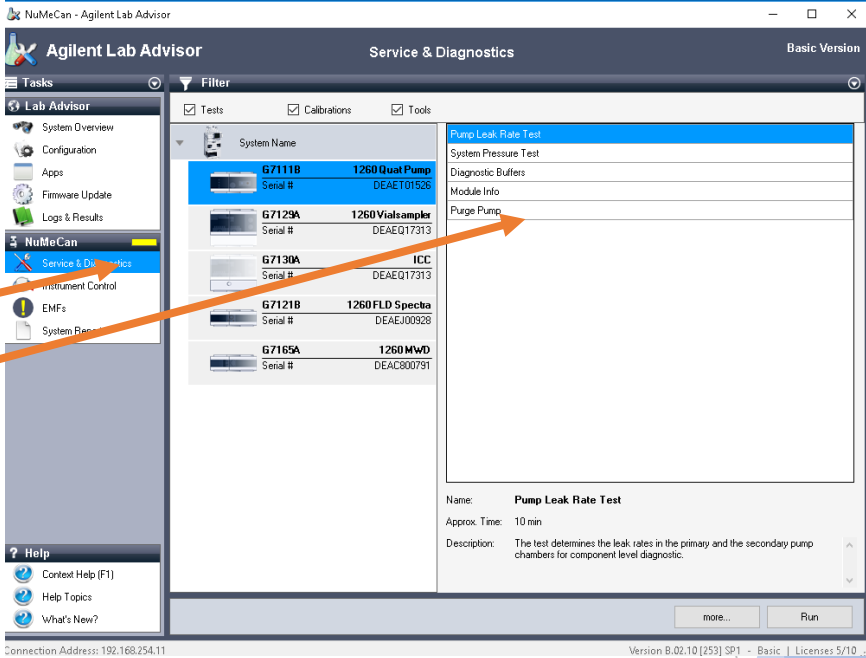
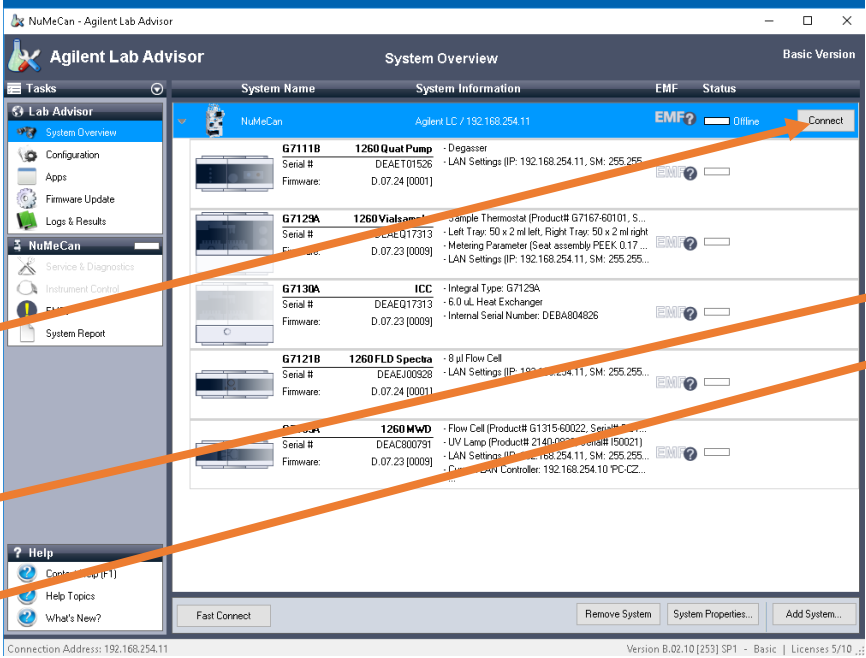


Connect

Service & Diagnostic

Purge Pump

Ouvrir la valve quand demandé



Avant chaque run



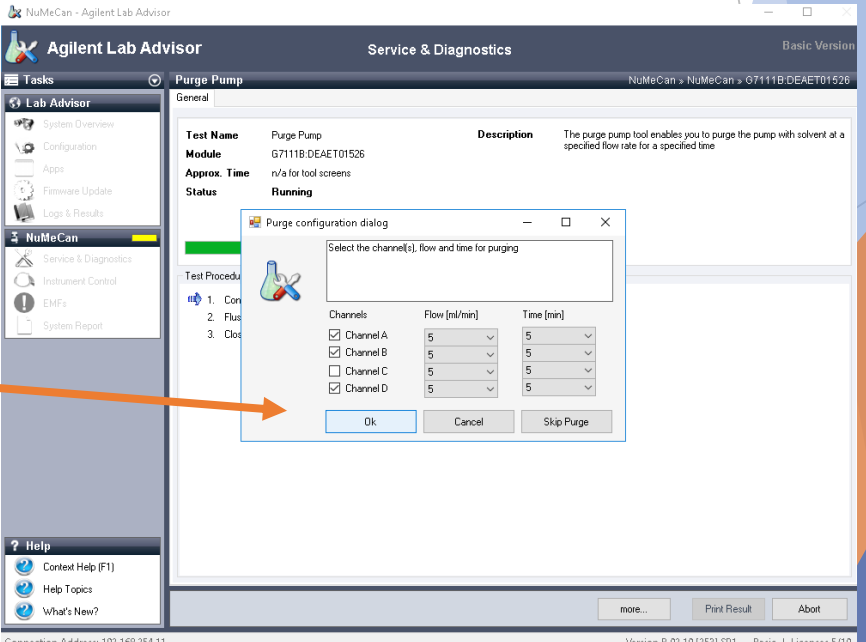
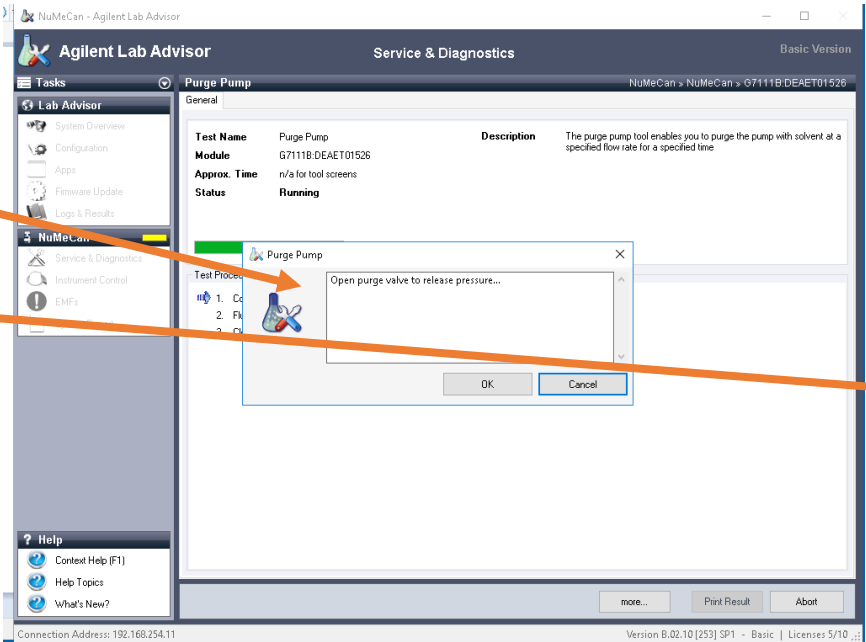
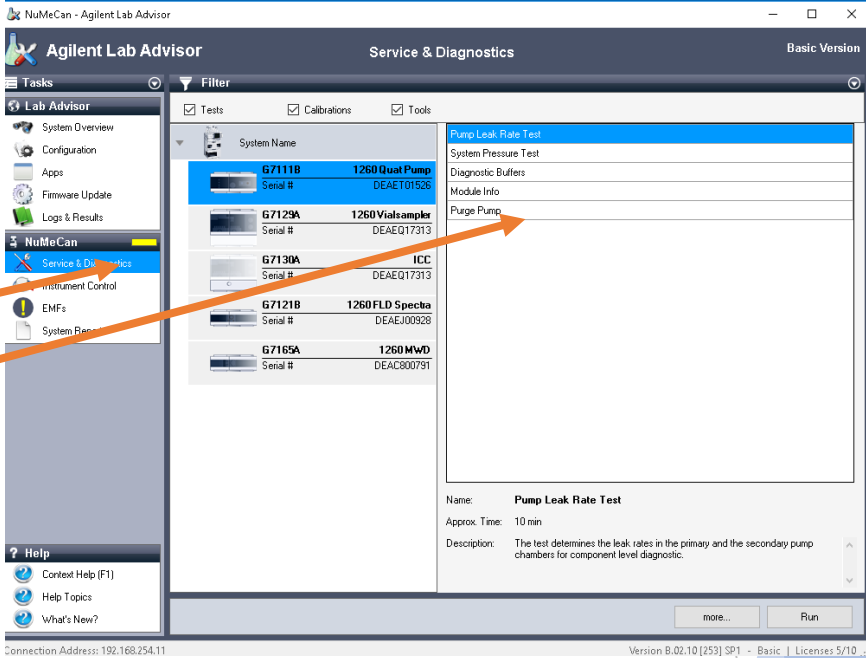
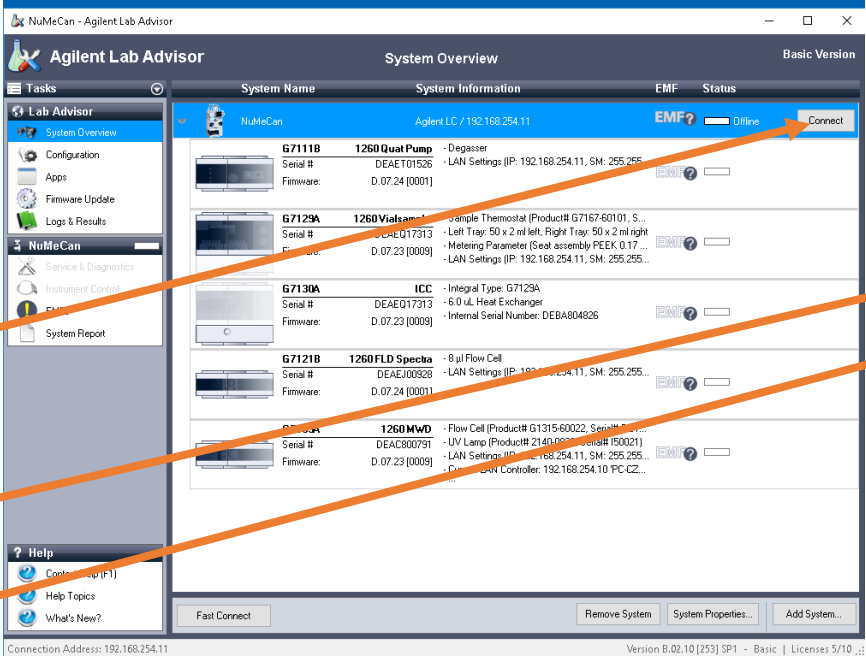
Connect

Service & Diagnostic

Purge Pump

Ouvrir la valve quand demandé

Cocher les bouteilles à purger.
5ml/min pdt 5 min



Avant chaque run



Connect

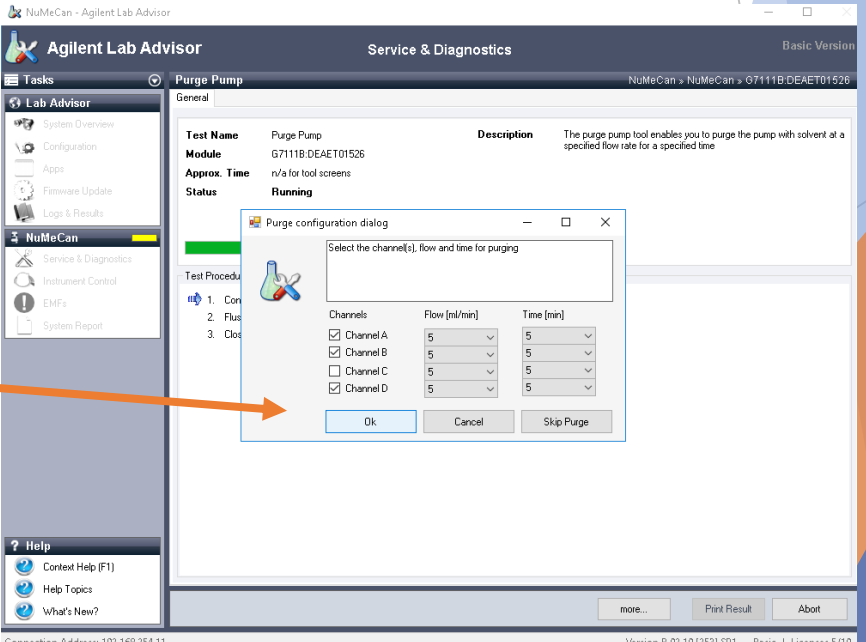
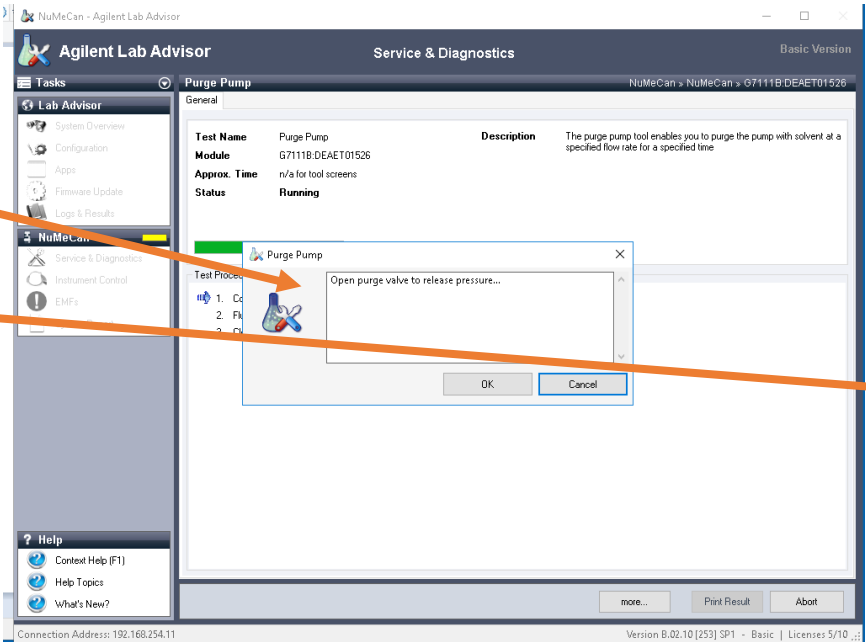
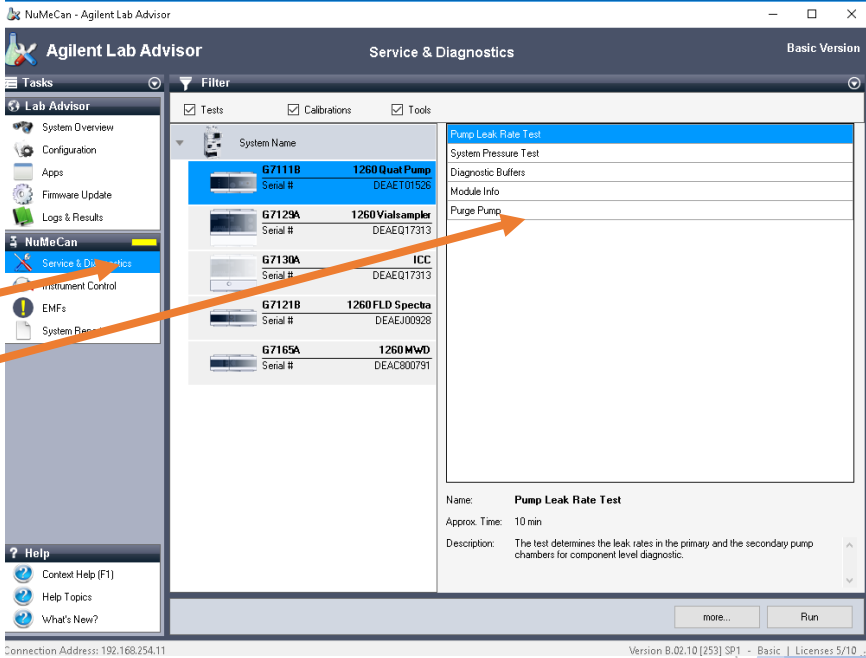
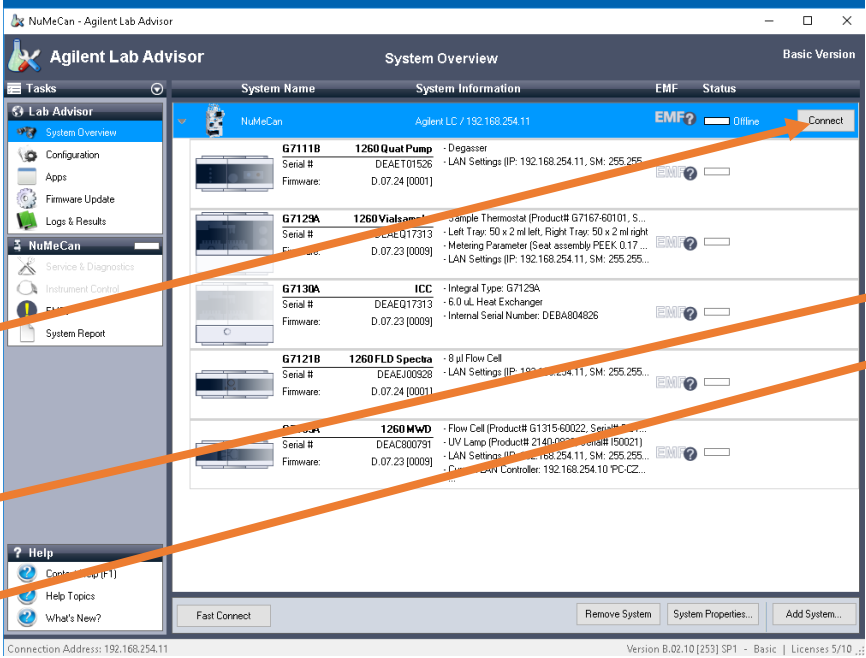
Service & Diagnostic

Purge Pump

Ouvrir la valve
quand demandé

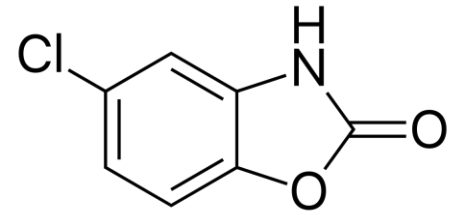
Cocher les
bouteilles à
purger.
5ml/min pdt
5 min

Refermer valve
quand purge
terminée

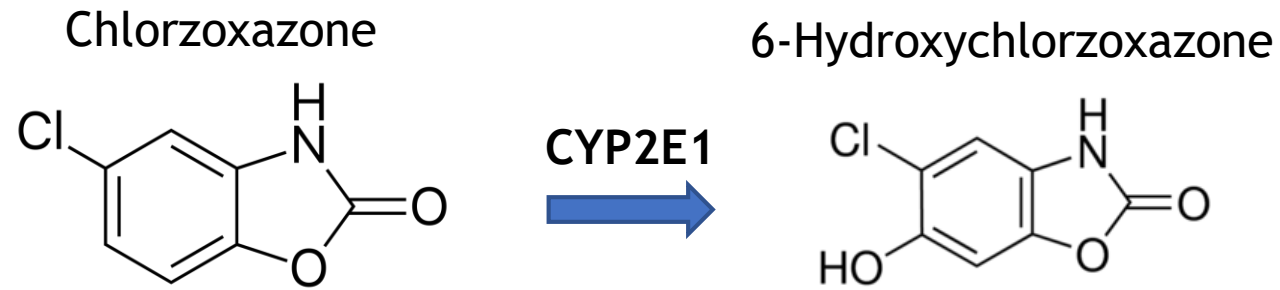


Evaluation de l'activité CYP2E1 par mesure de la métabolisation de la Chlorzoxazone

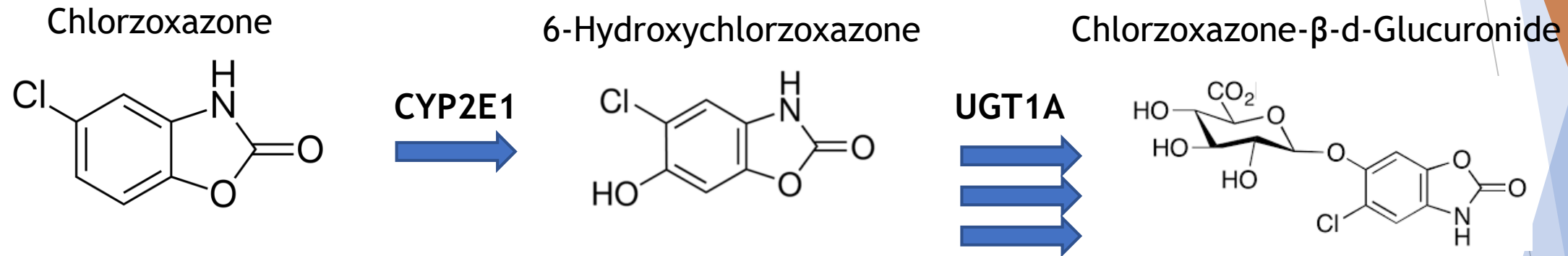
Chlorzoxazone



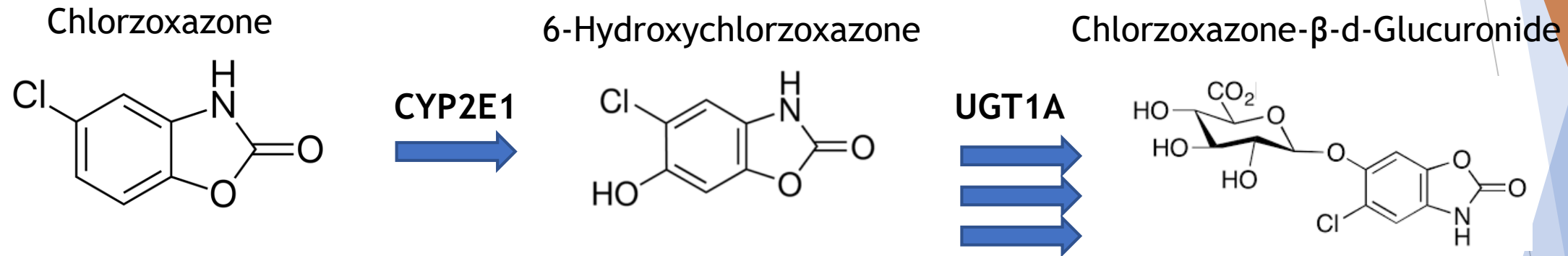
Evaluation de l'activité CYP2E1 par mesure de la métabolisation de la Chlorzoxazone



Evaluation de l'activité CYP2E1 par mesure de la métabolisation de la Chlorzoxazone



Evaluation de l'activité CYP2E1 par mesure de la métabolisation de la Chlorzoxazone



Dosage de la forme glucuronide donne l'information sur ce qui s'est formé en 6-OH, et donc une idée de l'activité du CYP2E1.

Il est aussi possible de reconvertir le O-Glc en 6-OH grâce à une enzyme, la β -Glucuronidase

Culture

-Cultiver les cellules (ex: HepaRG), faire les traitements/conditions souhaitées comme d'habitude.

Culture

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-Eliminer milieu de culture, laver au PBS, et remplacer par du milieu Williams **sans rouge de phénol** et sans suppléments (pas de SVF, pas de DMSO, pas d'antibio, hydro, insuline etc ...)

Le milieu contient 300µM de Chlorzoxazone

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→Le rouge de phénol peut perturber la lecture.

Et avoir moins de suppléments = moins de pics dans le profil HPLC = plus facile à analyser.

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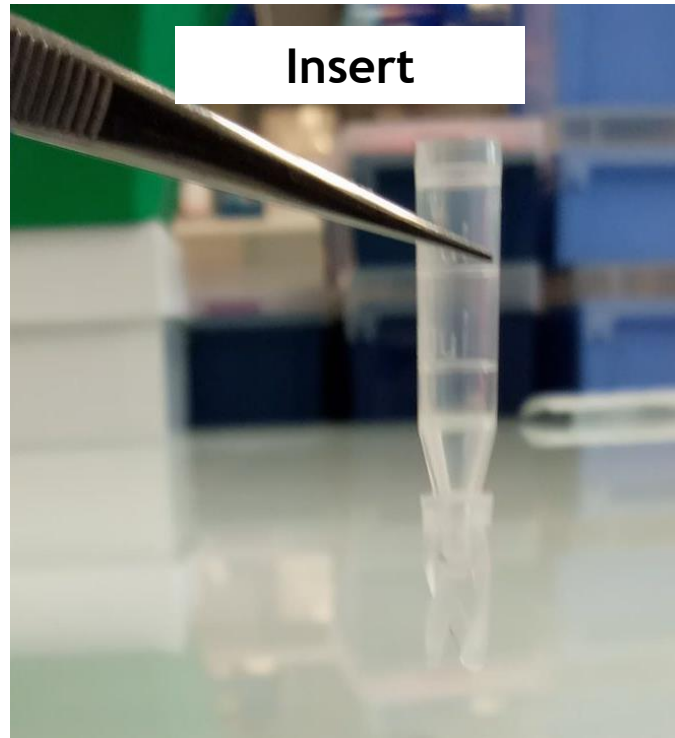
Et avoir moins de suppléments = moins de pics dans le profil HPLC = plus facile à analyser.

- Incubation 6h environs dans ce milieu.

- Récupérer les surnageants, centrifuger 10 min à +10 000 g pour éliminer les débris potentiels

- Congeler surnageants et cellules.

Echantillons

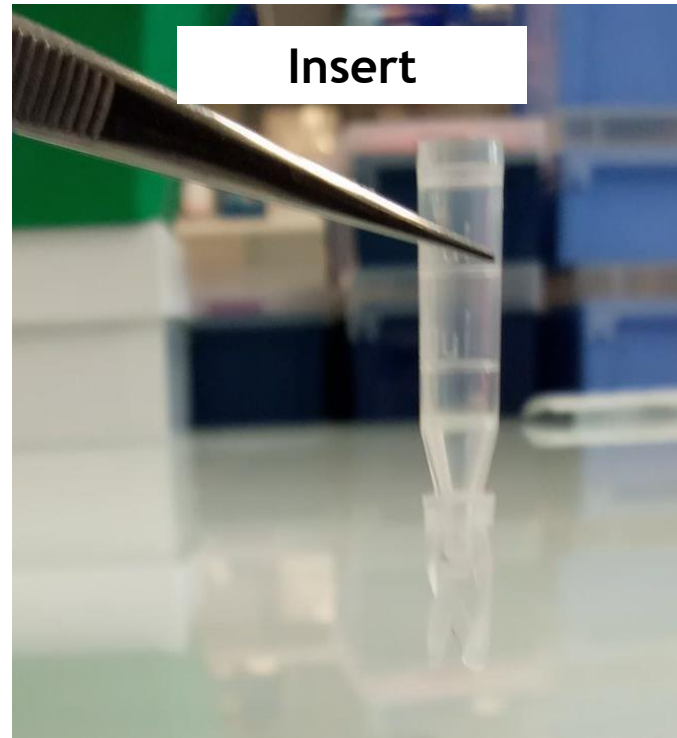


Echantillons

Eau



Premier run à blanc, vérifier propreté de la colonne.
Dernier run de lavage.



Echantillons

Eau



Premier run à blanc, vérifier propreté de la colonne.
Dernier run de lavage.

Standards:

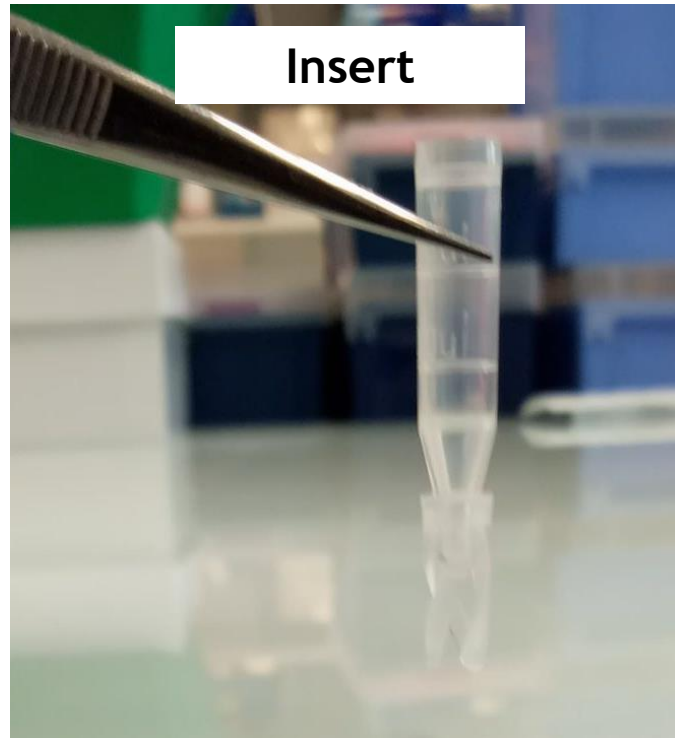


Identifier et quantifier les métabolites
(commencer à 300µM)

CHZ

CHZ-6OH

CHZ-O-Glc



Echantillons

Eau



Premier run à blanc, vérifier propreté de la colonne.
Dernier run de lavage.

Standards:



Identifier et quantifier les métabolites
(commencer à 300µM)

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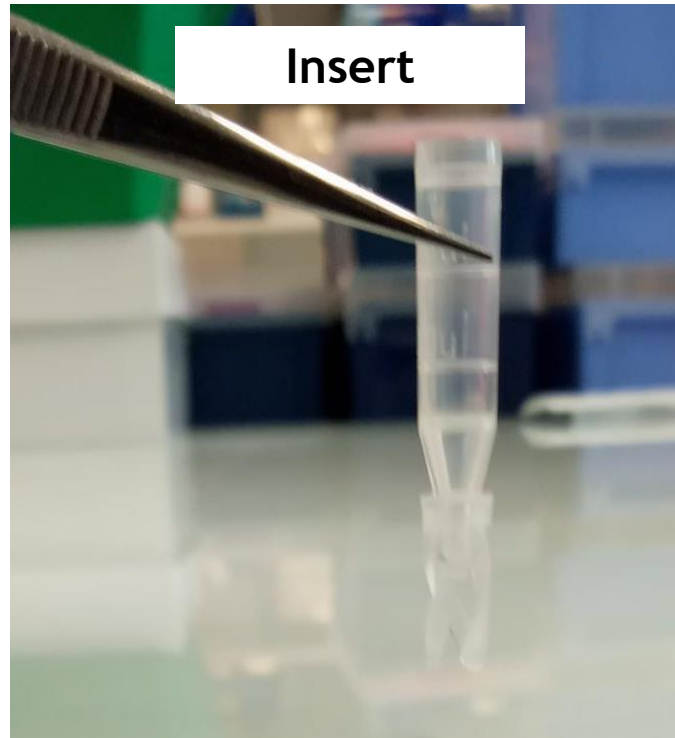
Echantillons:

X1

X2

X3

...



Solvants

- ▶ Acétonitrile
- ▶ Acide acétique (0,1%) + 0,25% Triéthylammonium (TEA)

Métabolites pas visibles sans le TEA, nécessaire de l'ajouter.

Méthode

► Injecteur

- 45 µl injecté
- Stop time à 30min
- Echantillons à RT (pas de réfrigération)

Méthode

► Injecteur

- 45 µl injecté
- Stop time à 30min
- Echantillons à RT (pas de réfrigération)

► Four

- Maintenu à 30° C

► Lecteur UV-Visible

- 287nm (± 16) Référence à 400nm (± 80 nm)

Méthode

► Injecteur

- 45 µl injecté
- Stop time à 30min
- Echantillons à RT (pas de réfrigération)

► Four

- Maintenu à 30°C

► Lecteur UV-Visible

- 287nm (± 16) Référence à 400nm (± 80 nm)

► Pompe

Temps (min)	% Acide Acétique	% Acétonitrile	Débit	Pression max
0	98	2	0,5ml/min	600bar
1	98	2	0,5ml/min	600bar
10	10	90	0,5ml/min	600bar
22	10	90	0,5ml/min	600bar
27	98	2	0,5ml/min	600bar
30	98	2	0,5ml/min	600bar

Méthode

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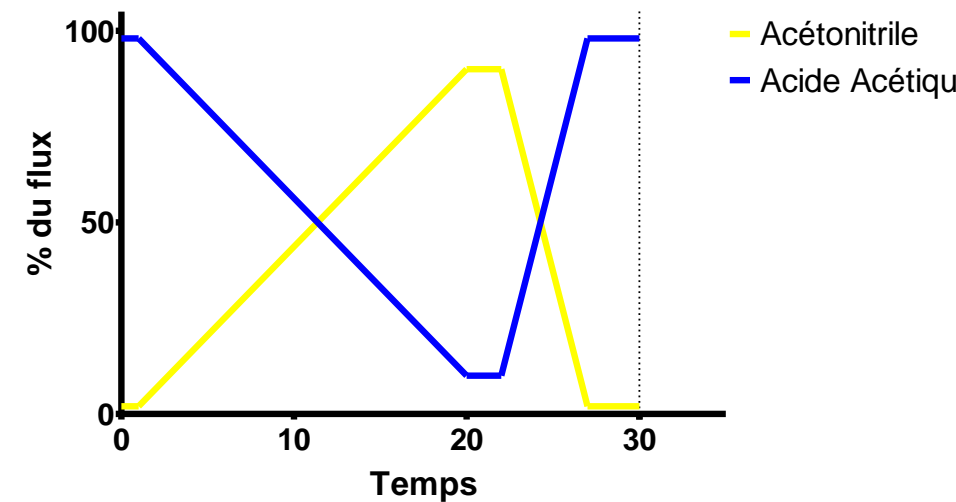
► Lecteur UV-Visible

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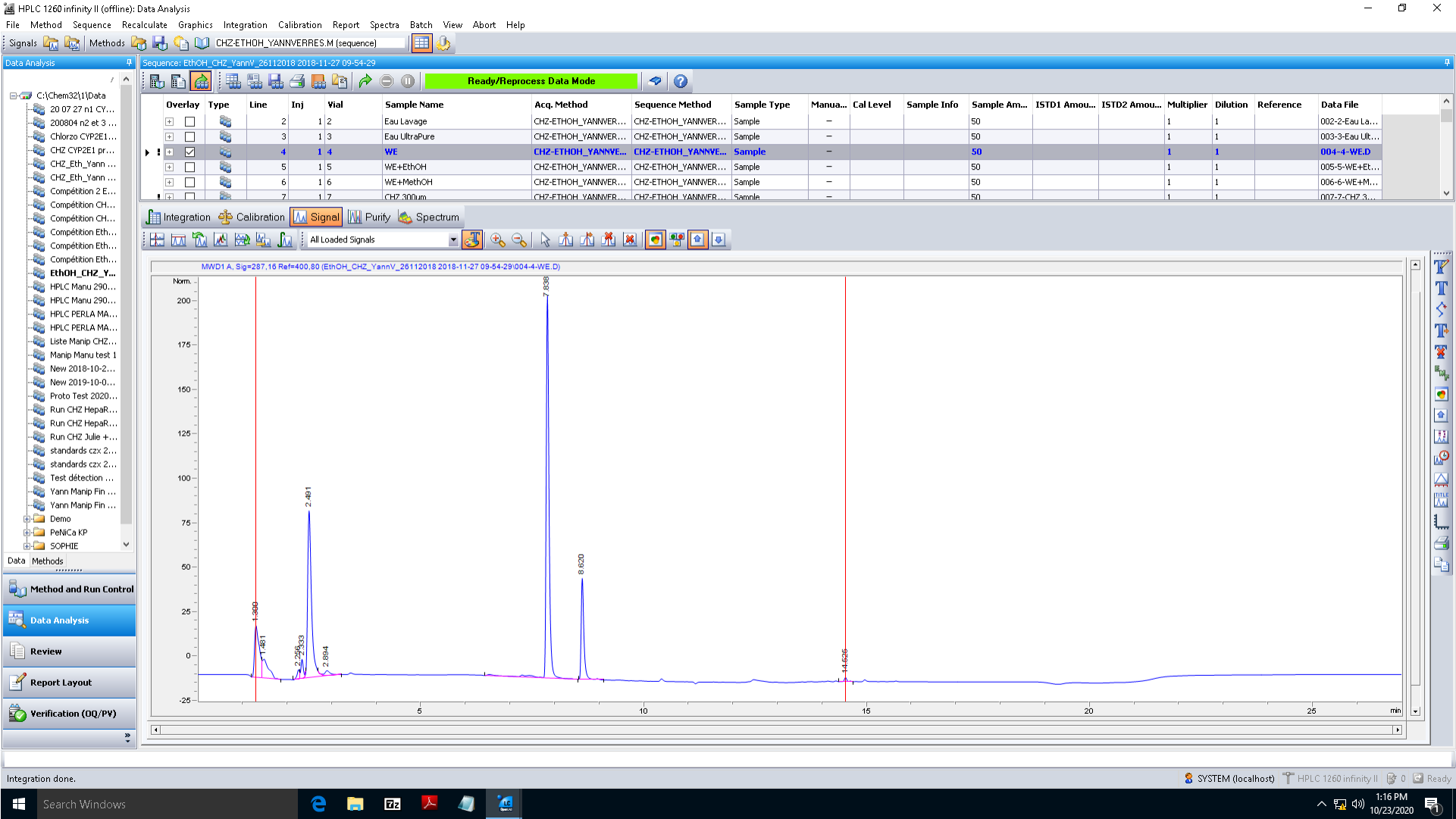
► Pompe

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27	98	2	0,5ml/min	600bar
30	98	2	0,5ml/min	600bar

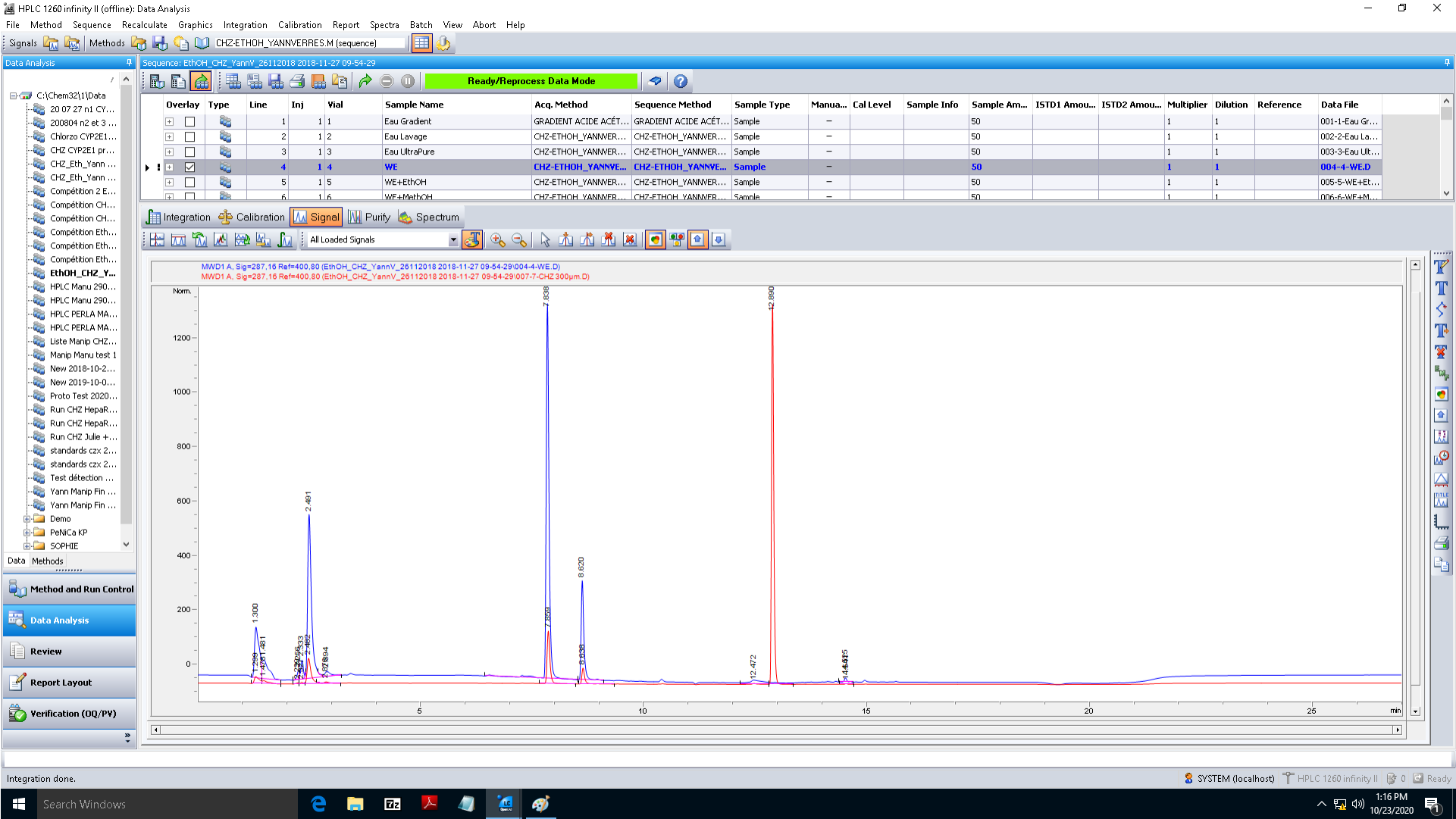
Flux des solvants lors du run HPLC



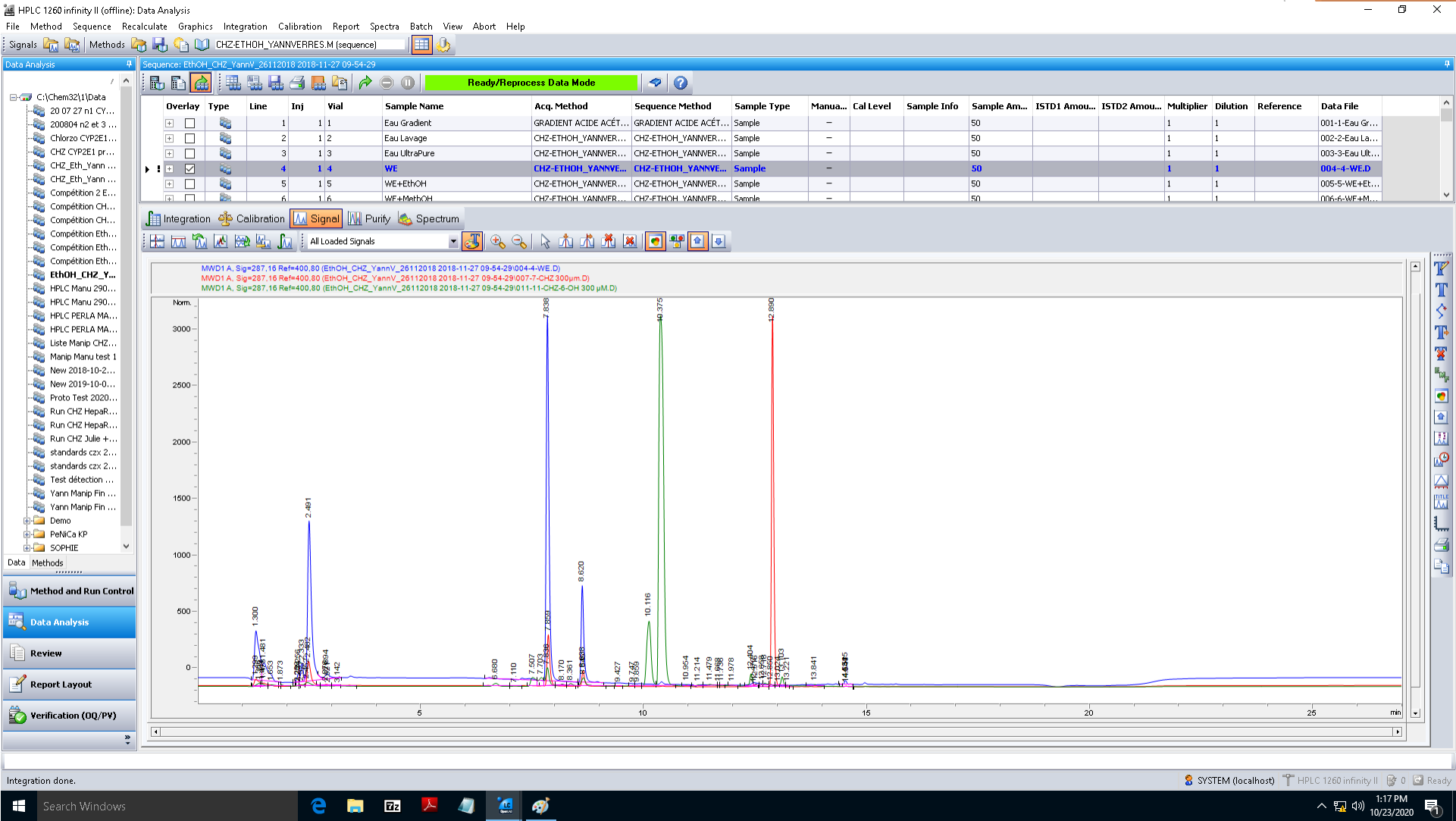
Résultats



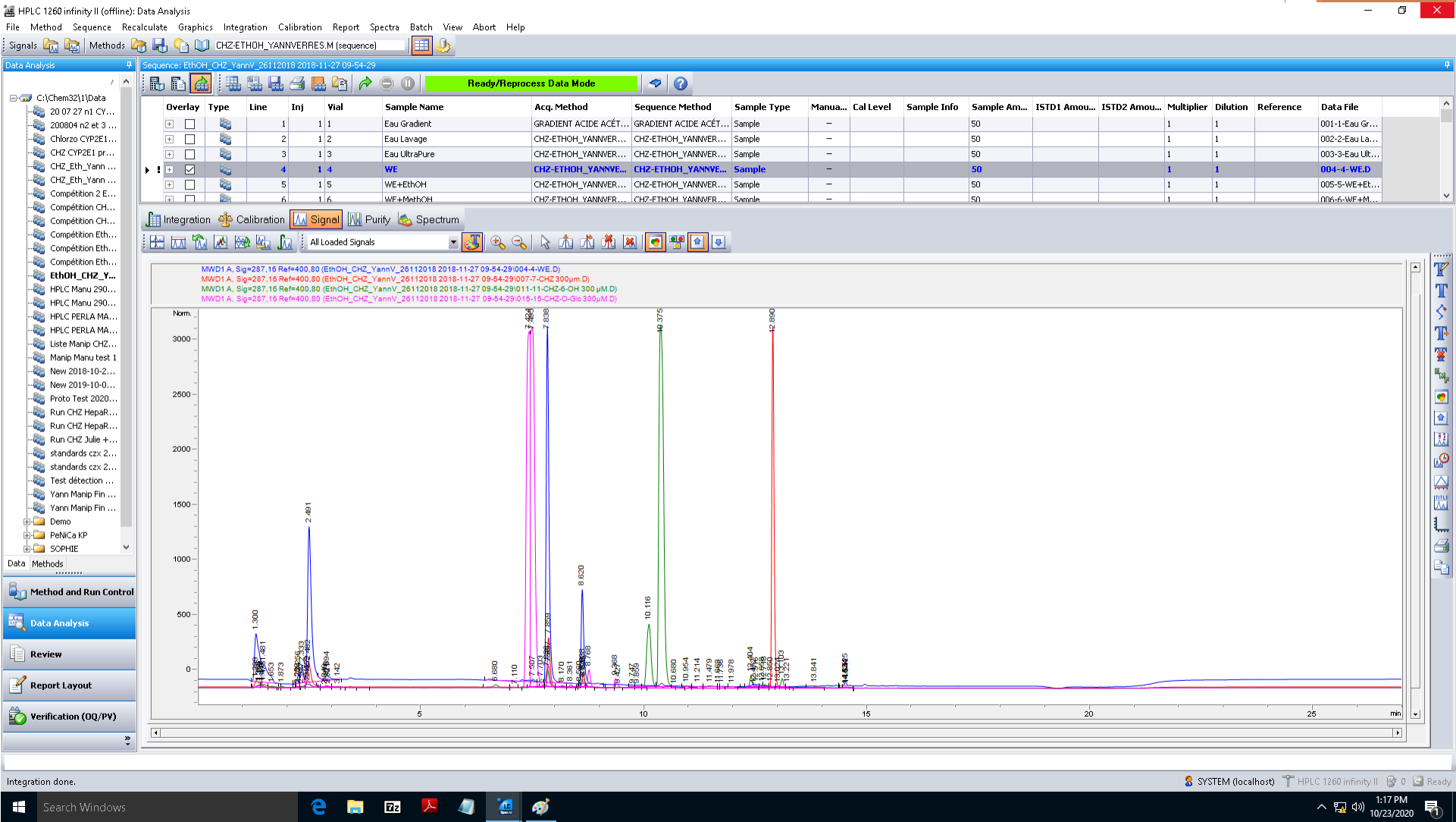
Résultats



Résultats



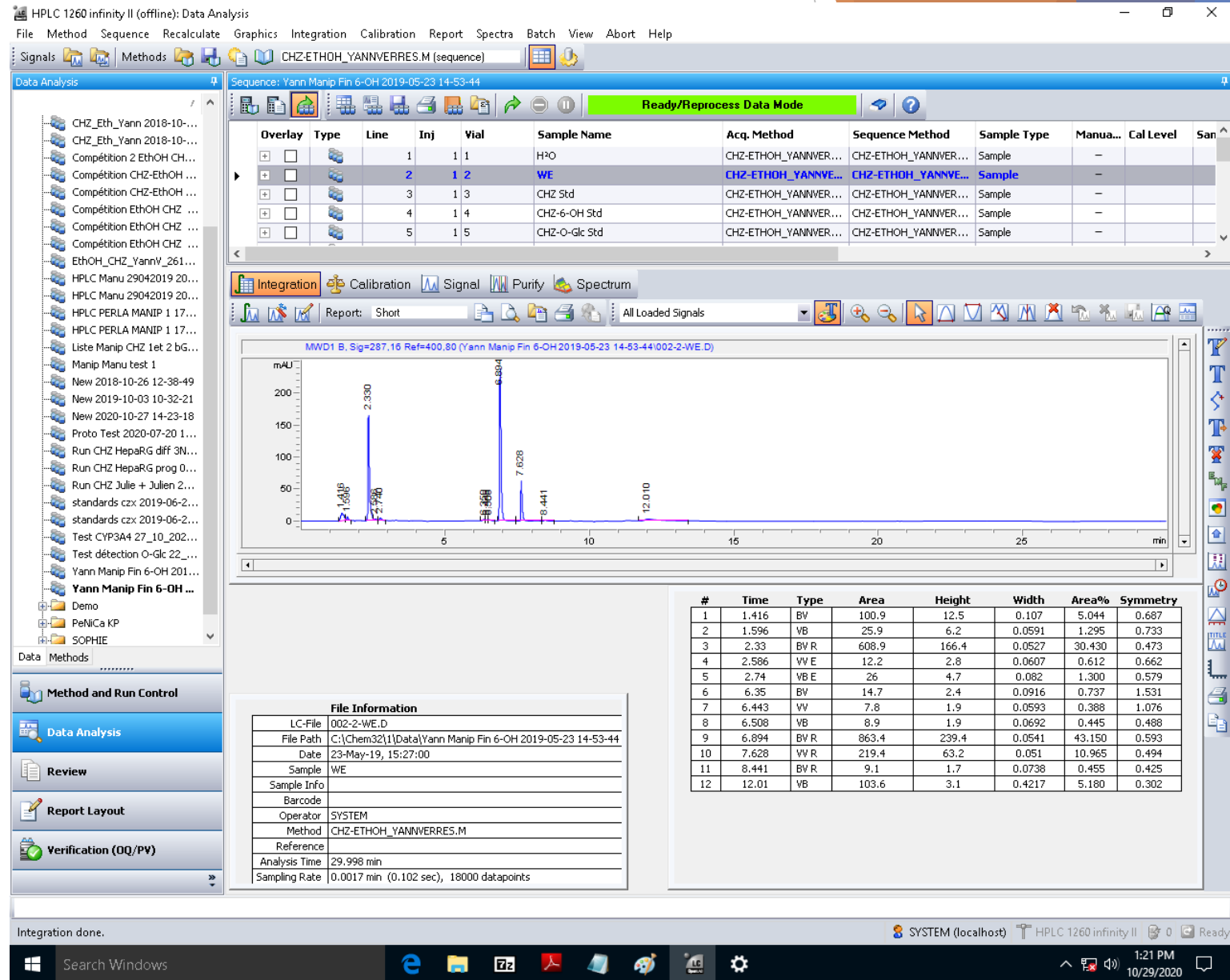
Résultats



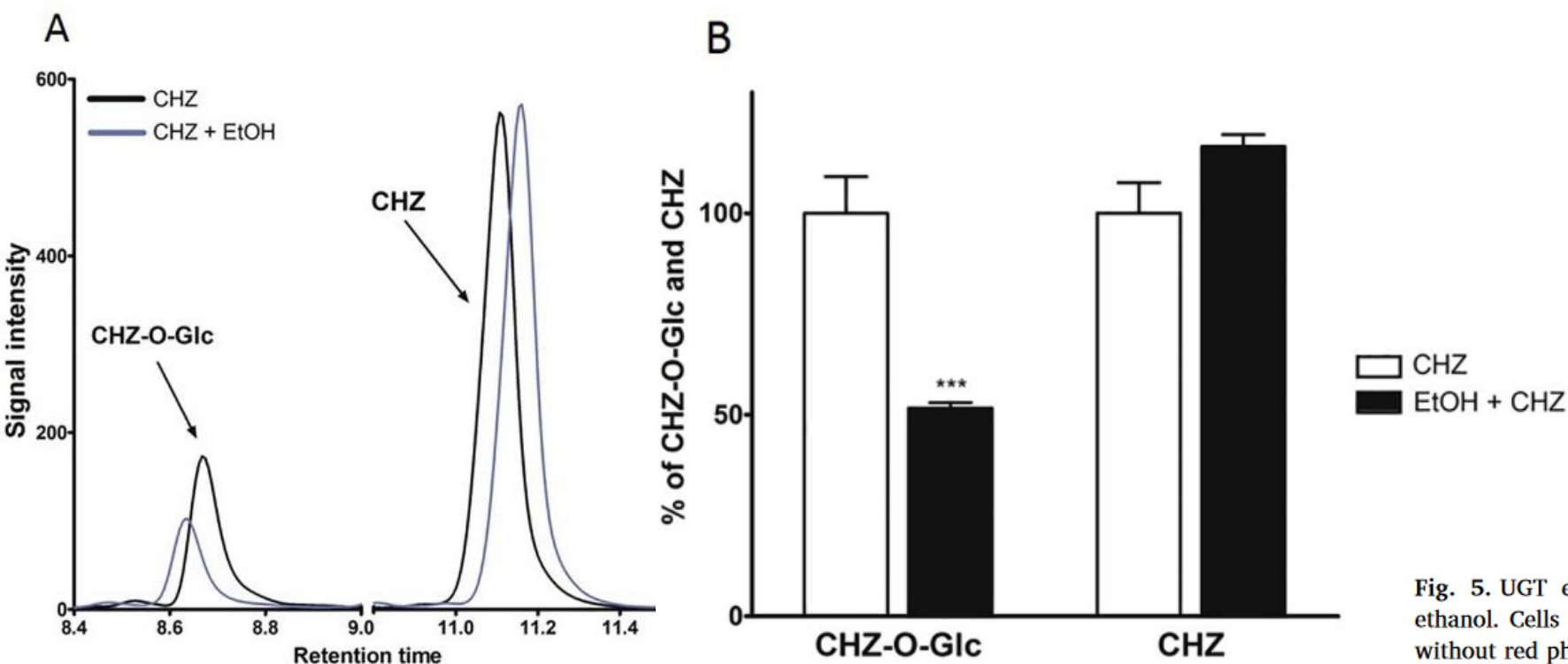
Résultats

- Profil HPLC exportable en format CSV (fichier texte) → A copier ensuite dans un tableur (Exel ou autre)
- Données d'intégration → copier le tableau et coller en document texte.

Temps du pic, valeur d'intégration, type de pic, largeur ...



Résultats



Hugbart and al. Toxicology in vitro, 2020

Fig. 5. UGT enzymatic activity in differentiated HepaRG cells treated by ethanol. Cells were incubated with incubation medium (William's E medium without red phenol) containing 300 μ M of chlorzoxazone (CHZ) or incubation medium (William's E medium without red phenol) supplemented with both CHZ (300 μ M) and ethanol (EtOH) at 50 mM for 4 h. The production of the CHZ-derived glucuronide (CHZ-O-Glc), reflecting UGT1A catalytic activity was analyzed by HPLC. Typical HPLC chromatograms (Fig. 5A) showing the peaks of CHZ-O-Glc and CHZ in culture media of cells incubated with CHZ and CHZ + EtOH. Peak area quantification of CHZ and CHZ-O-Glc (B) as the mean \pm SEM for three independent experiments performed in triplicate. Results are expressed as percent of control cultures (with CHZ) set at 100%. Statistics: *** $p < .001$ for cultures exposed to CHZ treatment compared with the cultures co-incubated with CHZ and EtOH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Merci pour votre attention !

