Waters AutoPurification System <u>Standard Operation Protocol</u>

Contents	5
A. User policy (Read before use!)	
i. Sample preparation	
ii. Mobile phase and columns	2
iii. During use	3
iv. After use	
B System overview	Δ
C Initialization	7
D. Analytical analysis	8
i. Set up LC and PDA method	9
ii. Set up MS method	
iii. Create sample list	
iv. View analytical data E. Preparative analysis	20
i. Set up LC method	
ii. Set up fraction collection method	
iii. Define fraction collection position	
iv. Create sample list	
V. View collection results	
F. Washing and shutdown	
G. Data transfer	
G. Data transfer	

A. User policy (Read before use!)

i. Sample preparation

- 1. Use 2 ml vials for sample injection.
- 2. Use 15 mL tubes with diameter of 16 mm for sample collection.
- 3. Label all your vials and tubes.
- 4. Dilute samples with starting mobile phase. (No absolute DMSO is allowed.)
- 5. Filter samples with 0.4 μm or 0.22 μm filter to remove precipitates.
- 6. The level of sample should reach the bottom line of vials (0.5 mL level).



7. Only flat-bottom and conical-bottom inserts with **<u>NO FEET</u>** are allowed.



ii. Mobile phase and columns

- 8. Only <u>reverse phase</u> chromatography is allowed.
- 9. Standard mobile phase (provided and refilled by CPOS)

Polar (A1): $H_2O + 0.1\%$ formic acid

Non-Polar (B1): ACN + 0.1% formic acid

- 10. NO running on 100% of A1 phase is allowed.
- <u>Analytical</u> column provided by CPOS: C18 5 μm, 4.6 mm I.D. x 50 mm length (located at analytic column 2)
- 12. **<u>Preparative</u>** column provided by CPOS: C18 OBDTM, 19 mm I.D. x 50 mm length (located at preparative column 1)
- 13. Bring your own guard, analytical and preparative columns for any deviations from our standard solvent composition and columns.

Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023

iii. During use

- 14. Log in PPMS tracker.
- 15. Create your own projects and save at D:\User Data\Department\PI. Do NOT modify others' projects or methods.
- 16. Make sure the run time of pump, PDA detector and QDa mass detector are consistent in their corresponding methods.
- 17. Stopping a run (1) is NOT recommended. After you stop the run, the next sample in the queue will NOT be injected but the LC will still keep flowing. Please manually stop the flow by

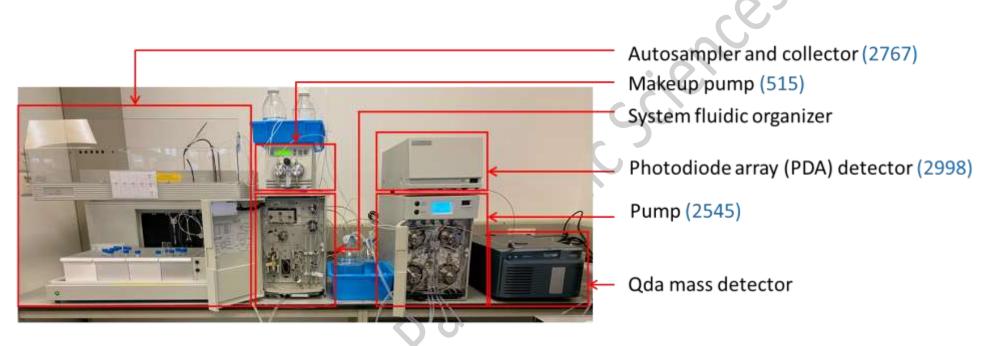


18. Stop the flow ($^{\text{Inlet Method}} \rightarrow ^{\text{C}}$) when you are still preparing samples or setting up methods to save solvent.

iv. After use

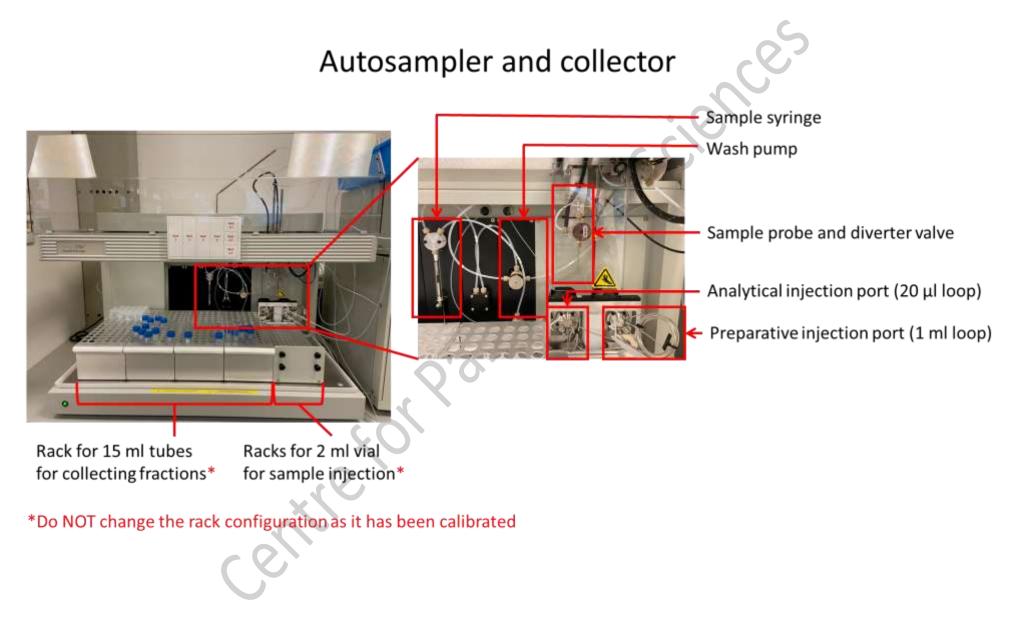
- 19. You MUST run <u>at least two blanks</u> (methanol) with "CPOS_analytical_wash" as the LC method and "CPOS_analytical_wash" as MS method (to monitor any contaminates left) to clean the column. (Part F)
- 20. You MUST <u>enable shutdown procedure</u> so that the flow, PDA detector and QDa mass detector will be automatically shut down after running all samples in the queue. (Part F)
- 21. Do NOT insert your own USB thumb drive to the PC connecting the instrument. Transfer data with CPOS USB drive and perform analysis on data transfer station (Part G)
- 22. Remove all your vials and tubes after use.
- 23. Log out PPMS tracker.

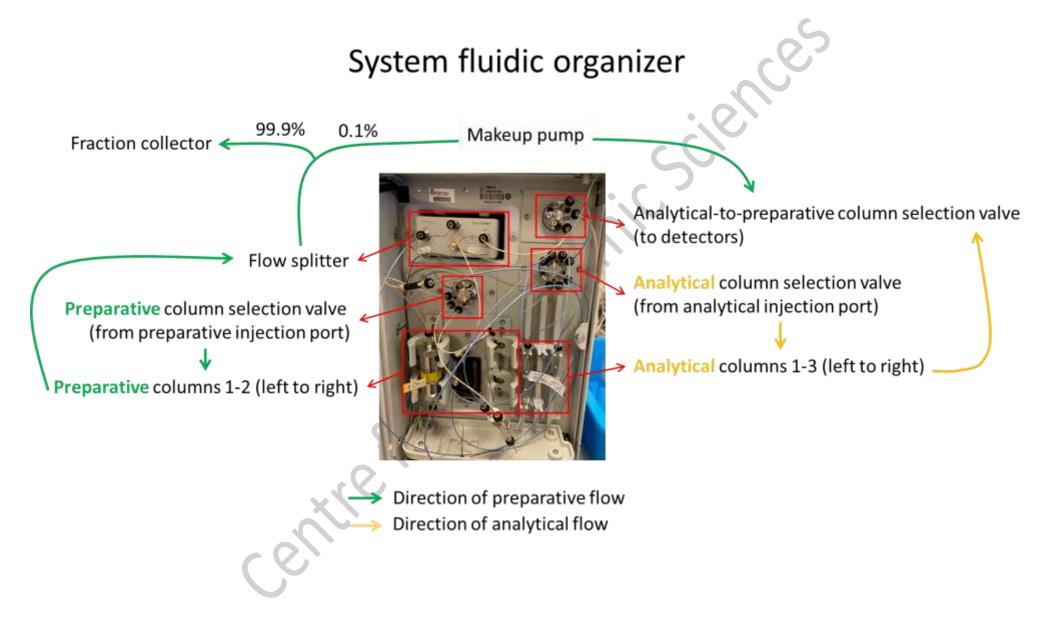
B. System overview



Number in brackets() represent its model no.

- entre



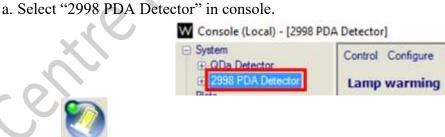


Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023

C. Initialization 1. Open the "MassLynx" software on desktop. Keep "MassLynx" software open; otherwise QDa mass detector will be disconnected. MS Console on "MassLynx" software. 2. Open the "acquity console" software by s electing 3. Initialize QDa mass detector. a. Select "QDa Detector" in console. W Console (Local) - [QDa Detector] System Control Configure QDa Dete 2998 PDA Detecto b. Select ^{Communic} to operate QDa mass detector. Green light on the top left of icon represents the detector is on. c. Wait ~1 minute for the green status light on QDa mass detector to change from flashing to solid. d. Make sure its status is "Ready" in console.

Ready	calibration	
	Status	Int. Calibrated
	Calibrant	Internal
	Calibrated On	June 23, 2020

4. Initialize PDA detector.

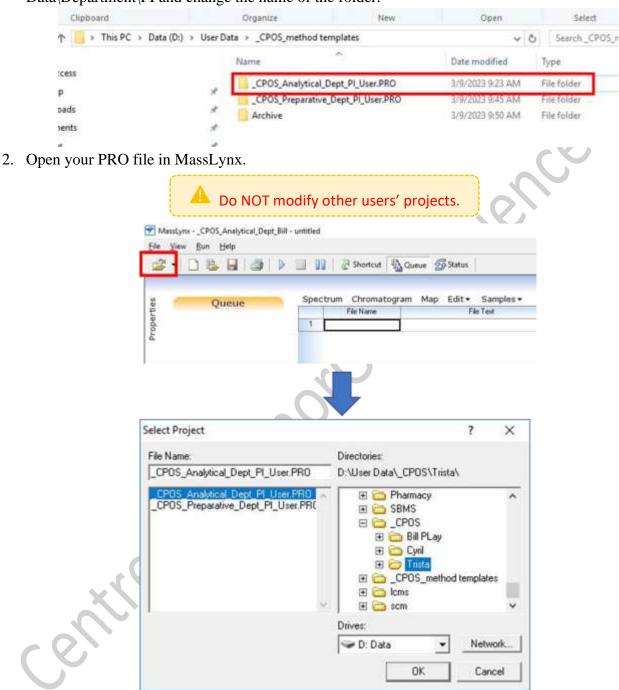


- b. Select to turn on the lamp of PDA detector. Green light on the top left of icon represents the detector is on.
- c. Make sure the light of lamp on PDA detector turns to green.



D. Analytical analysis

1. Copy the folder "D:\User Data_CPOS_Analytical_Dept_PI_User.PRO" to D:\User Data\Department\PI and change the name of the folder.



3. Edit File name, File text, bottle position and inject volume.

ľ

		Canas Status						
			q	beae Is Empty				
Spe	ctrum Chromato	prant Mag Edit + Samples +						
	Factione	file Text	Induit The	3.0%x	Traject Volanies	HOTH	HS Ture File	Sechi
1	Fisitione E-mpis		intel No. DRDS_anaphosi_tempiere	3/5/		HD Fau analytical_Mergitale	HS Ture File	See U
1 2		file fast			0.000.07405_		A Distance Street of	Sephil
1 2 3	Empli	The Tast (set both posttan and inact values)	DPOS_analytical_template		0.000.07425_ 0.000.07425_	analytical_temptake	A Distance Street of	Sech D

Set up LC and PDA method i.

4. In the sample list, right click the inlet file name and select "Edit".

Image: Solvert Selections Set up LC flow condition by selecting Set up LC f			Q	ueue Is Empty		
Image: Construction of the second	_	h	nlet File	Bottle	Inject Volume	
ODS_modeled_wash Example of the second o			1000000			CPC
Inter method will be shown in the window below. Flow on/off Load previously saved LC met LC flow condition Image: Construct the solution of t		Concernance of the second s		Browse		PC
Set up LC flow condition by selecting Vertex Vertex Set up LC flow condition by selecting Vertex		and a statistic over the ball of the ball of the ball of the ball		Edit		Sec.
Intermethod will be shown in the window below. Flow on/off Lamp on/off Load previously saved LC method LC flow condition Image: Converter of the shown in the window below. LC flow condition Image: Converter of the shown in the window below. LC flow condition Image: Converter of the shown in the window below. PDA parameters Image: Converter of the shown in the window below. Set up LC flow condition by selecting Image: Converter of the shown is provided by CPOS. A1: Water + 0.1% formic acid Image: Converter of the shown is provided by CPOS. A1: Water + 0.1% formic acid Image: Converter of the shown is provided by CPOS. A1: Water + 0.1% formic acid Image: Converter of the shown is provided by CPOS. A1: Water + 0.1% formic acid Image: Converter of the shown is provided by CPOS. A1: Water + 0.1% formic acid Image: Converter of the shown is provided by CPOS. A1: Water + 0.1% formic acid Image: Converter of the shown is provided by CPOS. A1: Water + 0.1% formic acid Image: Converter of the shown is provided by CPOS. A1: A1 (A2) A Image: Converter of the shown is provided by CPOS. Chromatographic Pump Image: Converter of the shown is provided by CPOS. A1 (A2) A Image: Converter of the shown is provided by						_
Add User Cler Selected Set use Conserverter. 287 Vial Converter. 287 Vial Converter. <t< td=""><td>_</td><td></td><td></td><td>1</td><td></td><td>-</td></t<>	_			1		-
Intert Intert Intert Intert <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td></td<>						
Clear Selected Customize Display AutoSampler Bed Layout 277 Vid Converter Converter.						
Let method will be shown in the window below. Flow on/off Lamp on/off Lad previously saved LC met Flow on/off Lamp on/off Lad previously saved LC met Flow condition of the shown in the window below. LC flow condition by selecting Inter PDA parameters PDA parameters Flow condition by selecting Inter Set up LC flow condition by selecting Inter PDA parameters Selections' as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid						f cX
Autosampler Bed Layout. 2767 Vial Converter Inlet method will be shown in the window below. Flow on/off Lamp on/off Load previously saved LC met U flow condition in the window below. C flow condition is the window below. PDA parameters is the window below. Set up LC flow condition by selecting Inter Set up LC flow condition by selecting Inter Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid B1: ACN + 0.1% formic acid Chromatographic Pump Run Tame: 14.00 min Moble Phase Events Analog Solvent Selection A and Solvent Names Pressure Limits Low: Descure Lim						
Z757 Vial Converter Inlet method will be shown in the window below. Flow on/off Load previously saved LC met LC flow condition Image: Convert Selections and D1 if you use the solvents provided by CPOS. At: Water + 0.1% formic acid B1: ACN + 0.1% formic acid D1: ACN + 0.1% formic acid						
Inlet method will be shown in the window below. Flow on/off Lamp on/off Load previously saved LC met I C flow condition is the initial formation of the initial formation						
Flow on/off Lamp on/off Lamp eviously saved LC met LC flow condition Image: Constrained on the second					in the second seco	1
LC flow condition LC flow condition Here Autosampler parameters Here PDA parameters Here		File View Tools	LC Waterstein Quade A	for Help	d previously	saved LC met
LC flow condition Het Autosampler parameters PDA parameters Water 2000 Ket up LC flow condition by selecting Inlet Set up LC flow condition by selecting Inlet Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid B1: ACN + 0.1% formic acid Chromatographic Pump Run Time: 14.00 min Mobile Phase Events Analog Solvent Selections A1 and Solvent Names Pressure Limits bar				0.00		
LC flow condition		Status		i Schert Levels		
Autosampler parameters PDA parameters Water 2000 Set up LC flow condition by selecting Intet . Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN	LC flow or			Putgo	A	31
Autosampler parameters PDA parameters Waters 2000 Set up LC flow condition by selecting Inlet . Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid B1: ACN + 0.1% formic acid Chromatographic Pump Run Time: 14.00 min Mobile Phase Events Analog Solvent Selections Solvent Names Pressure Limits Low: D bar	LC flow co			Time Initial 0.4	6 (A) mos	
Autosampler parameters PDA parameters Waters 2000 Set up LC flow condition by selecting Inlet . Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Run Time: 14.00 min Mobile Phase Events Analog Solvent Selections A CA1 CA2 A A1 Cov: D bar		Inset		05 million 11		5
PDA parameters Witter 2000 Set up LC flow condition by selecting Inlet . Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Noble Phase Events Analog Solvent Selections A: CA1 CA2 A: A1 CA2 A:	Autosampler para	meters \longrightarrow 🥑		The participant of the	12	E)
Set up LC flow condition by selecting Inlet . Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Run Time: 14.00 min Mobile Phase Events Analog Solvent Selections A: A1 CA2 A: A1 Solvent Names Pressure Limits Low: Dar		Autosampler		Pressaria att	ā ao a	6
Set up LC flow condition by selecting Inlet . Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Run Time 14.00 min Mobile Phase Events Analog Solvent Selections A: A1 CA2	PDA para	meters —> 🕑		Note Burning	1.17	
Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Nobile Phase Events Analog Solvent Selections A: A1 C A2 A: A1 C A		Waters 2998		Hole haveg		-10
Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Nobile Phase Events Analog Solvent Selections A: A1 C A2 A: A1 C A		40				
Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Nobile Phase Events Analog Solvent Selections A: A1 C A2 A: A1 C A	Set up LC flow c	ondition by selecting	g Inlet			
A1: Water + 0.1% formic acid B1: ACN + 0.1% formic acid Please inform our staff in advance if you use your own solvents. Chromatographic Pump Run Time: 14.00 min Mobile Phase Events Analog Solvent Selections A: © A1 © A2 A: A1 Chromatographic Pump Nobile Phase Events Analog Chromatographic Pump A: © A1 © A2 A: A1 Chromatographic Pump Chromatographic Pump Ch	-	•	-	use the solve	nts provide	d by CPOS.
B1: ACN + 0.1% formic acid ▲ Please inform our staff in advance if you use your own solvents. Chromatographic Pump Mobile Phase Events Analog Solvent Selections A: CA1 CA2 A: A1 A1 Curve Curve Cur			-		•	-
Chromatographic Pump Bun Time: 14.00 min Mobile Phase Events Analog Solvent Selections A: A: A1 A: A: A1 A:						
Mobile Phase Events Analog Solvent Selections Solvent Names A: A1 C A2	A Plea	se inform our staff i	in advance i	f you use you	r own solve	nts.
Mobile Phase Events Analog Solvent Selections Solvent Names A: A1 C A2	Chromatogra	phic Pump		В	un Time: 14.0	0 min
Solvent Selections Solvent Names Pressure Limits A: • A1 • A2 A: A1	and the second se		1		00000000000000000000000000000000000000	
A: A1 C A2 A: A1 Low: 0 bar					Designed	
	All So		100 000 000 000 000 000 000 000 000 000		5	
B: @ B1 C B2 B: A2 High: 276 bar	A	(A1 C A2 A:	IAI		Low:	bar
	B	@ 81 C 82 8	A2	•	High:	276 bar

8. Set the maximum system pressure at 276 bar or 4,000 psi.

Chromat	tographic Pump		Run Time: 14	00 min
	Mobile Phase Events A	Analog		
444	Solvent Selections	Solvent Names	Pressure	Limits
5000	A: (• A1 (A2	A: A1	▼ Low:	0 bar
- -	B: @ B1 @ B2	B: A2	▼ High:	276 bar

9. Set the gradient table with the following as an example for C18 column (50 mm long). Set flow rate at 1-2 mL/min for analytical run.

If the length of your own column is > 100 mm, reduce the flow rate to 0.5 - 0.8 mL/min to reduce the pressure.

	Time (min)	%A	%В
Sample injection	Oth	95	5
	1st	95	5
Gradient elution	10 th	0	100
Column washing	12 th	0	100
Column regeneration	13 th	95	5
	14 th	95	5

10. Make sure the "Run Time" of pump (at top right-hand corner) is consistent with that in the gradient table. If they do not match, the "Run Time" of pump will be prioritized.

hromat	tographic	Pump				Run 1	Time: 14	.00 min	
1 1	Mobile Pha	se Events	Analog						
₩ •.•	A: @	A1 C A2	A: 🗚		- -]]	Pressure Low: High:	Umits 0 bar 276 bar	
T.	Gradier	nt 🙃 Syst	tem 🤆 Pump	Only	s	eal Wash	Period:	5.0 minu	te
 	Gradier	Time (min)	Flow (mL/min)	Only %A	S %8	eal Wash Curve	Period:	5.0 minu	te
	Gradier	Time	Flow		1	1	Period:	5.0 minu	te:
	Gradier	Time (min)	Flow (mL/min)	%A	%B	Curve	Period:	5.0 minu	tes
- -	1	Time (min) Initial	Flow (mL/min) 1.00	%A 95.0	%B 5.0	Curve	Period:	5.0 minu	tes
- 7	1 2	Time (min) Initial 1.00	Flow (mL/min) 1.00 1.00	%A 95.0 95.0	%B 5.0 5.0	Curve Initial 6	Period:	5.0 minu	tes
	1 2	Time (min) 1.00 10.00	Flow (mL/min) 1.00 1.00 1.00	%A 95.0 95.0 0.0	%B 5.0 5.0 100.0	Curve Initial 6 6	Period:	5.0 minu	tes

Select "Analytical" in "Analytical/Prep Valve" and the appropriate analytic column number (1-3, from left to right) in "Analytic Selection Valve". The analytical column provided by CPOS (C18 5 μm, 4.6 mm I.D. x 50 mm length) is located at analytic column 2.

	Analytical/Prep Valve:	sector advectories	tic Selection Val	ve:	Colum	n Name:	
u	Analytical	Anal	lytic Column 2				•
	-Initial Switch States -	_	(d)	_			
	1: Off	• 3:	Off	٠	5:	Off	•
	2: Off	• 4:	Off		6	Off	•

12. Set the "Initial Flow Rate" of 515 pump (makeup pump) to 0.00 mL/min because it will not be used in analytical run.

M	515 Pump (A) Initial Row Rate:	Solvent Name:	Pres	sure Limits:	
10	0.00 mL/min		• Low	0	bar
			High	414	bar
<i></i>	B Initial Row Rate:	Solvent Name:	Pres	sure Limits:	
	0.00 mL/min		Low		bar
		Solvent Name:	- Low		

- 13. Select ^{Autosampler} to set up autosampler parameters.
- 14. Select "Left loop 20 μl" for analytical run. Keep other parameters as default as the graph below.
 Waters 2767 Autosampler

op
op Aspiration Speed (%) 20
4 Dispense Speed (%) 20
4 Dispense Speed (%) Air Gaps Air Gaps



15. Select Waters 2998 to set up the PDA parameters.

- a. **Lamp**: make sure the lamp is ON.
- b. <u>**3D data**</u>: Enable 3D data for a full scan.
- c. λ range: The range of full can be 190 800 nm. Signal out of this range cannot be detected.
- d. <u>**Run Time**</u>: Make sure it is consistent with that of the gradient table (step 7). The PDA detector will NOT detect or record any signal if it stops before the gradient stops.
- e. Keep other parameters as default as the graph below.

98 PDA Deter	Ctor d. Run 1	Time: 14.00 min
amp: a		?
D data: b	Enable	
λrange: C	190 to: 800 nm	
Resolution:	1.2 • nm	
ampling rate:	10 v points/sec	
iter time constant:	Nomal 💌 0.2000 sec	
xposure time:	Auto 🚽 msec	
ptions;	Interpolate 370 nm line region	
	☑ Interpolate 656 nm line region	
Comment:		

16. (Optional) If you know the wavelength of UV absorbance of your target(s), set a specific channel for it (maximum 8 channels) for quantitative analysis.

2998 PDA Detector	Run Time: 10.00	- 0
General 20 Channels Analog Out Events	Hun Time. 110.00	min
Data mode λ I Channel 1 Absorbance ▼ 254 □ Channel 2	2 nm resolution	?

17. Select 🖬 to save your LC method.

18. Equilibrate LC method by selecting "Load Method". It will start to flow the initial step of the LC method. Monitor the pressure of the pump until it becomes stable (fluctuating +/-50 psi), which takes 5-10 min. Meanwhile, you can move to the next step.

	And the second s		_	_		
19	Status					
	Status Additional Statu	s Solvent Levels				1.1
Status	- Indicators	Pumps				
	Running	Time (mins):	0.00	a	0.0 %	
Inlet	O Pump On			6	0.0 X	
	Inject Cycle	Flow (ml/min)	0.00			
Ð	Ready			G	0.0 %	
tosampler	. ок	Pressure (bar)	1.4	8	0.0 %	
	Detector Scan	Mode: Idle				
iters 2998	oçan.	Milde, ide				

ii. Set up MS method

19. Edit MS method by right clicking the MS file in sample list.

	Bottle	Inject Volume	MS File	MS Tune File	S-ample Group
		0.000 CPDS_analytics	al_template	1000	
	5.3.8.F	0.000 CPOS_analytic		Browne	1
	5.3.0.F	0.000 CPOS_analytics	al_wash	Edit	
		0.000		Cut	
		0.000		Copy	
		0.000		Paste	-
		0.000		Add	
		0.000		Insert	
		0.000		10000	
× \		0.000		Clear Selected	
		0.000		Customize Display,	
		53 - 53		AutoSampler Bed Layout	
				2767 Vial Converter	
Experiment Setup - di\user da File Edit View Options To			er.pro\acqud	b\cpos_analytical_templa	ste.exp
File Edit View Options To		s Help	er.pro\acqud	b\cpos_analytical_templi	ite.exp

- 20. Select MS Scan to set MS scan parameters.
 - a. <u>Mass (m/z)</u>: The maximum MS scan range is 100 1000 m/z. Mass < 100 may have much noise while that > 1000 may give no signal.
 - b. <u>Time (Mins)</u>: Normally set consistent with gradient table but can also specify a shorter timeslot.
 - c. <u>Method</u>: Select whether ES+/ES- mode you will use.
 - d. <u>Cone Voltage</u>: the cone voltage needs to be optimized. The higher the mass to charge ratio, the high the cone voltage required. Recommended to start from 15 for ES +ve and 30 for ES -ve.

Ma St	ass (m/z) art 100	Method Ionization Mode ES+ ~
Er	d 1000	Data Continuum ~
). Tir St. Er		Cone Voltage Cone Voltage (V) 15
		OK Cancel

21. (Optional) Select SR (Selected Ion Recording) to specify target mass to obtain a chromatograph with higher sensitivity for quantitative analysis (maximum 32 SIR channels). You can set specific time slot to increase sensitivity.

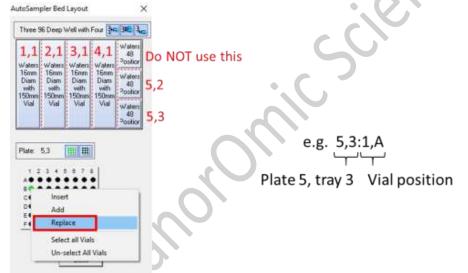
	Method		Chanr	nels			
	Ionization Mode	ES+ 🗸		Compound Nam	Mass (m/z)	Cone (V)	Comments
			1	example	120	20	
	Retention Window	(Mins)					
	Start	0					
	End	10					
22. Select	la to save	your MS	meth	od.			

iii. Create sample list

1. In sample list:

	a.	b.	с.	d.	e.	f.	
Spe	Fie Name	Gram Map Edit • Samples • File Test	Iniet File	Botte	Inject Volume	MS File	MS
1	Sample	[set bottle position and inject volume]	CPOS_analytical_template		0.000 CPOS_	analytical_template	
2	Compileopulation 1	The min woodning (Rejnet work (Q).	CROS_seal_local_insth	1,205	0.000.0205	analytical minite	
3	Conpulsory Wash 2	15-min washing (Inject vol:0)	CPO5_analytical_wash	5,38,F	0.000 CPOS_	analytical_wash	
1.4					10/2010		

- a. <u>File Name</u>: sample name. Only include alphabets and "_". Maximum 20-30 characters, otherwise may generate errors.
- b. <u>File Text:</u> fill in any note related to the sample or setting.
- c. Inlet File: load your established Inlet method file.
- d. <u>Bottle</u>: the position of sample vial. Right click \rightarrow "AutoSampler Bed Layout"



- e. <u>Inject Volume</u>: maximum 20 μ l for analytical run, but recommend to start with 5-10 μ l to prevent contamination of the system.
- f. MS File: load your established MS method file.
- g. Other columns can be left blank.
- 2. Right click sample list to add more samples.
- 3. Select to save your sample list.

Before running your sample, check the following:

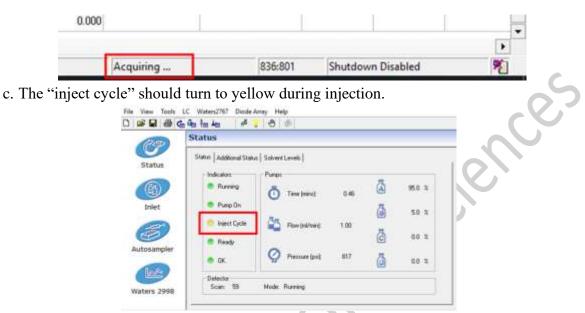
- The pressure of the pump is less than 2000 psi. If it is >2000 psi, call our staff.
- The pressure of the pump is stable (fluctuating within +/-50 psi).
- No leakage of solvent from the two extremes of column(s)
- The sample vials are in the right position as defined in your sample list.

Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023

4. Highlight the samples to be run and select

to start the run.

- 5. Monitor the status of instrument.
 - b. The status (at the bottom right of Masslynx software) should have changed from "Instrument Present" or "Not scanning" → "waiting for injection" → "acquiring"



6. You can view the sample list going to be run in "Queue". You cannot edit the running queue but can delete or add a queue or allow a queue to be run first.

P		23 to 2	Sample 23 Acquiring		
Current queue	Queue	Spe	ctrum Chromatogram Map Ed File Nane	It + Samples + File Test	MS
		1	20190909	7.66 7.64	
ore	✓ <	2	2019909 Black01		PW Local HDV
	Patrick_2019	1	2079909 Black02		PW Local HDV
		4	2019909_Blark03		PW Local HEIM
		5	20190809_Trial_10mm		PW Local HDM
		6	20130905_Trial_10nin_carpover		PW/Local HDX
	Patrick_2019	7	20190909_Trial_30s		Pe/Local HDV
		- A	Services Trail Stratement		26.11.++11.0%

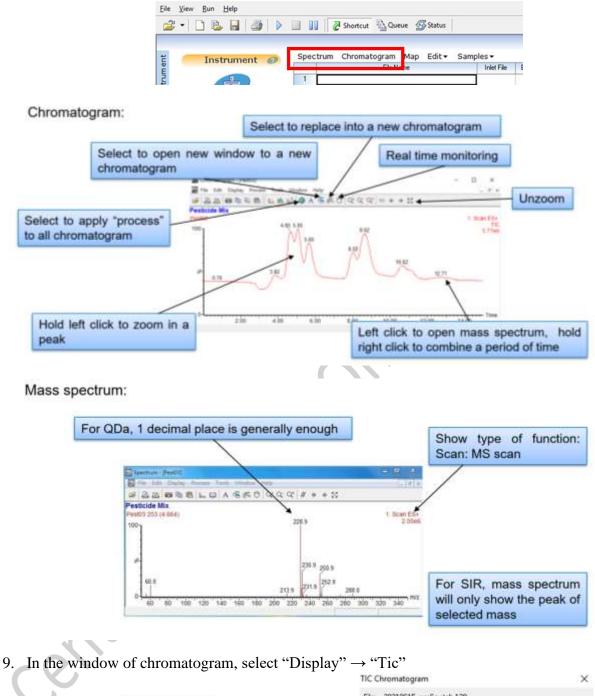
7. If you want to stop run immediately, you can click the red square on the top of Mass Lynx.



Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023

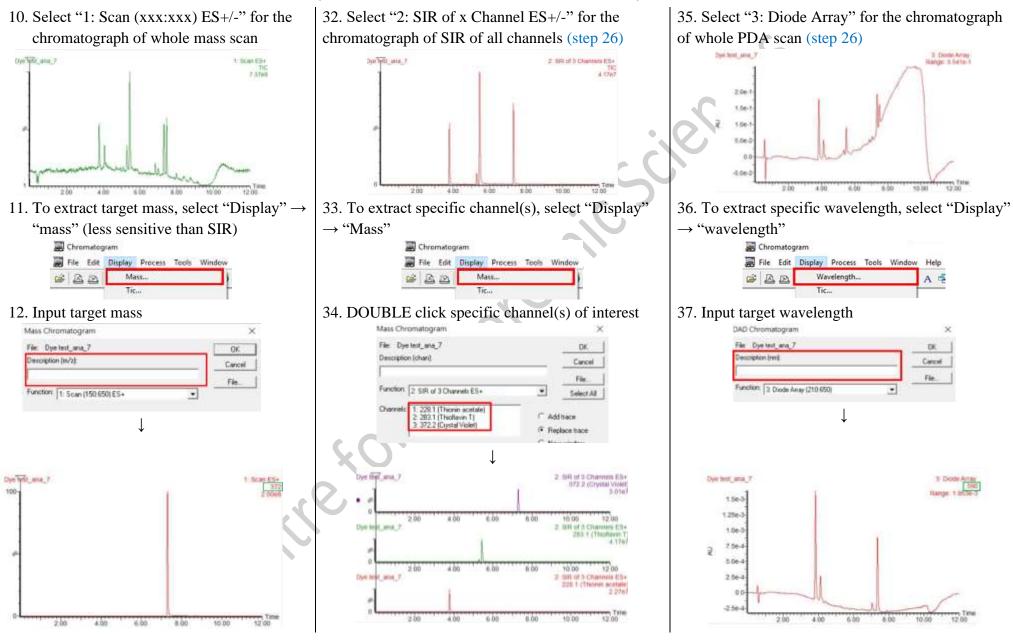
iv. View analytical data

8. Select the sample of interest and then select "Spectrum" or "Chromatrogram" to view data.



File Edit	em Display Process Tools Win	low Help Run	Function	OK
* 8 B	Mass Tic	ASSO	2. SIR of 1 Channel ES+ hy106a 3. Diode Array [190:800]	File
20210615_wr 100-	Remove		•	
	Real-Time Update ChroTool DDATool		IF Additace ☐ BPI Chromato ☐ Replace trace	igram
	DDATeel_		C Replace trace C New window	

Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023



38. To find the peal	area, select "Process"	" \rightarrow "Integrate".
----------------------	------------------------	------------------------------

File	e Edit	Display	Process	Tools	Window	Help	Run
r 1	A.D.	160 B	Inte	grate			

39. You are recommended to select "Automatic noise measurement".

Chromatogram N	loise	Integrate]
Peak-to-peak an	nplitude 900000		
-		Сору	
Smooth	Enable smoothing	Paste	
Smooth Peak detect	Enable smoothing ApexTrack Peak Inte	Paste	

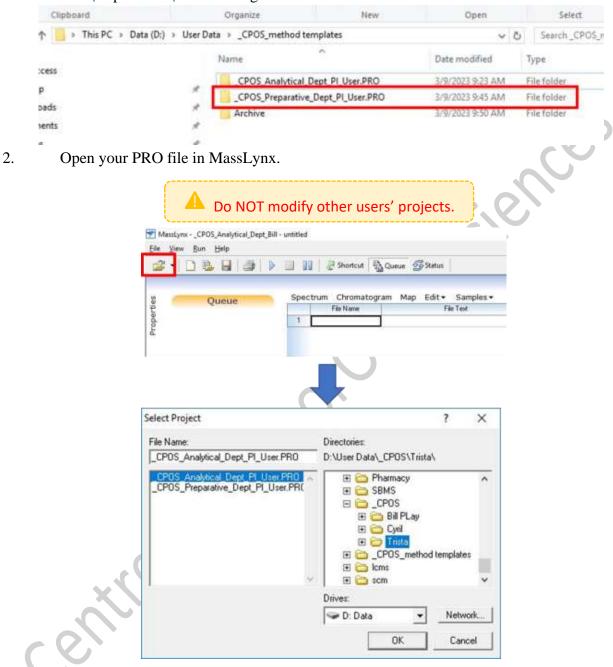
40. If the peak area did not appear after integration, select "Display" → "Peak Annotation" and select "peak response area".

Display Process Tools Window Mass	Chromatogram Peak Annotation	
Tic Analog Remove Real-Time Update ChroTool DDATool PDATool Range > Pointer View Fraction Display	Annotation Type Peak Top Time Peak Top Scan Peak Punty Decimal Places Scan Base Peak Mass Decimal Places Peak Response Area Decimal Places Peak Response Height Symmetry	Annotation Threshold C % Full Scale 0.0 Intensity 0 C All Peaks Level High BioLynx Component Label Digest Label Scan Set Mass Decimal Places
Fractions by tube Peak Annotation Customize Toolbar		OK Cancel

41. You must wash the column and system and enable shutdown after use, please refer to part F. Washing and shutdown (P.32) for details.

E. Preparative analysis

1. Copy the folder "D:\User Data_CPOS_Preparative_Dept_PI_User.PRO" to D:\User Data\Department\PI and change the name of the folder.



3. Edit File name, File text, bottle position and inject volume.

- 6	ngis 52 bington (PC										
1.1	a di sunce i da	an Sites									-
			Quantum In	Contraction of the							
			Queue 19	centled.							
		n Maj 201+ Sangha-	Queue 19	r staffely.							
	Taber	n Mapi Edit+ Sampler+ Ineffe	144	Figst I Maile	Factor (Factor)	e. 1816	Taxas De	Peril	(March	West Aug 11	Validade Partici
			Querce 19	rys/15aie	Parts Feaster	e site 201, poetin (inde	Tata la (75) year	Massill.	March 1	Verdages.	Vaccopil Para
	Taber		144	Figst I Maile	Passa (Passete)		Hadas file (793 jagas	Pead.	Marik 201	109-6.44(81) 2.00 2.00	theory I Carlos EXCONST. 4
	Taber		Line Actio	Figer Filmine RE RE	Paola: (Feaster	(PDL provide a state	Tata fa O'Ejrepe	Pecci.	Anali 201	1004 AU(0.1) 1-00 1-00	LIND Mar. 4

Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023

4	Change	1	1. formeration	"Duan quatizza?"
4.	Change	the samp	le format to	"Preparative".

Edit +	Samples -		() () () () () () () () () ()		-
	Add			C:\MassLynx\	OK
preparative_b				dioxin.tmt	Cancel
reparative_v	Delete			Dioxin_EPA1613	Lance
eparative_v	Fill			diverse.FMT	Browse
	Clear	٠		FractionLynx Preparative	
	Column			Quantity	
	Format	٠	Customize	Spark.fmt	
_	Sort	•	Load	Standard Addition Experiment StdAddSampleListFormat1.fmt	
	Number of Samples		Save	designed and the second s	
	Number of Injections			Description	
	AutoSampler Bed Layout			Preparative	
	2767 Vial Converter				

i. Set up LC method

1. In the sample list, right click the inlet file name and select "Edit". Inlet method will be shown in the window below.

Inlet File	Bottle Inject Volume Process	Param		templeta.bgm, CPO5_prep LC Waters2767 Diode A	lenay Help	2 butteringive	Utern_	5 (R.)
POS_preparative_terminist	15 31 0 100 000				16 0			
POS_preparative_wa	Browse			Status				
05_preparative_wa	Edit		C	CPUM LES				
	Cut		Status	Status Additional Statu	a Solvent Levels			
			Sector Com	- Tedicators	Purei			
	Сору			O Furning			ä	100.5
	Paste		and the second se	C Peop On	The (sm)	0.00	-	
	Add		Intes		14			0.013
	Insert			 Next Eacle 	Row Individual	0.00		
			Autosampler	· Ready			G	30 2
	Clear Selected				Ø Person bat	1.0	.72	40.1
	Customize Display			• or.			19	100.3
				Detector				
	AutoSampler Bed Layout		Waters 2998	Scan Hode ide				
	2767 Vial Converter			-				
_			Fur Help, press F1					



- 2. Select Inlet to set up LC flow condition.
- 3. Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS. A1: Water + 0.1% formic acid
 - B1: ACN + 0.1% formic acid

Chromat	ographic Pump		DATE	14.00	min
Galoma			Fun Lin	ne: 14.00	min
1	Mobile Phase Events /	Analog			
8.4.6	Solvent Selections	Solvent Names	Pr	essure Limits	
JULL	A: CALCA2	a. [A1		ow: 0	bar

Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023

4. Set the maximum system pressure at 276 bar or 4,000 psi.

nionia	tographic Pump		nu	n Time: 14.00	min
	Mobile Phase Events /	Analog			
	Solvent Selections	Solvent Names		Pressure Limits	
out	A: (A1 (A2	A: A1	···· ··	Low: 0	bar
	B: @ B1 @ B2	B: A2	•	High: 276	bar

5. Set the gradient table with the following as example. Set flow rate at maximum 10 mL/min for preparative run.

	Time (min)	%A	%В
Sample injection	0 th	95	5
	- 1 st	95	5
Gradient elution	7.5 th	0	100
Column washing	8.5 th	0	100
Column regeneration	9 th	95	5
alart i	10 th	95	5

If you wish to translate the **analytical** LC gradient table to **preparative**,



Open "Columns Calculator" (Columna on desktop.

Type in the **analytical** LC gradient table on the left, then a **preparative** LC gradient table will be automatically generated on the right.

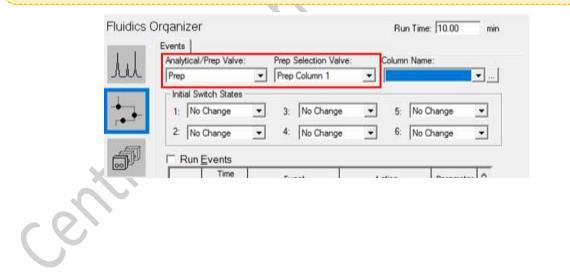
Autor State	0	Analy	ytical					Pri	eparati	ve		56
Fran. Insuite per Colorier System Mathed	ingen nelled ingelit regelster rek ingelster rek ingelster ingelst	1000 en 10 en 10 en 1000 en 1000 en 1000 en 1000 en 1000 en	D				1997 - 299 Selation - 299 - 29	ne to Nill nil S nil Lossi S ne to sei ne sei S ne				
	-	-				0.00						0.0
	and the last		10000	-		Galactic Salarian	And Address			-	-	
		184	1.166	185	94	.108	1248.	10,700			1.86	
100	1.000		44	144		144		144400	1000		144	114
10	1006	104	10	- 14	34	10	12.63	1448	100		14	1.0
18	1306	104	10	3	82.	100	1.00	7168	140 141 141		14	128
18 80 700	1006		100	1	34 32 34 34	128	2.00 2.00 2.00		100 101 100		1004	
1.0	1308	101 101 101	tand tand	11111	82 . 84	104	1.00	108	100			24
18	1000 USBN 1000 USBN	10 11 12 12	inte inte inte	1	82 34 34	101 107 101	1 100 1 200 1 100 1 100	7058 1056 1068	14 167 164		1014	24 34 34

1	lobile Pha	se Events	Analog							=
	Solvent	Selections -	Solven	t Names -			Pressure L	ints -	-	
	A: 6	A1 (A2	A A	1			Low:	0	bar	
	8 6	B1 ⊂ B2	8: Ā	2			High:	276	bar	
		Time (min)	Flow (mL/min)	%A	%8	Curve	î î			
<u>a</u>				%A	%8	Curve	n n			
	1	true al	10.00	95.0	5.0	10mail				
	2	1.00	10.00	95.0	5.0	6				
	3	7.50	10.00	5.0	95.0	6				
	4	8.50	10.00	5.0	95.0	6				
	5	9.00	10.00	95.0	5.0	6				
	6	10.00	10.00	95.0	5.0	6	v			
	1						1.51			

6. Make sure the "Run Time' is consistent with the gradient table.

Select "Prep" in "Analytical/Prep Valve" and the appropriate preparative column number (1-2, from left to right) in "Prep Selection Valve". The preparative column provided by CPOS (C18 OBDTM, 19 mm I.D. x 50 mm length) is located at preparative column 1.

If it is **wrongly** set as "<u>analytical</u> column", the analytical column may break due to unaffordable high pressure!



 Make sure "Initial Flow Rate" of 515 pump (makeup pump) is set to 1 mL/min. The high-pressure limit should be set at 276 bar or 4000 psi. Make sure the setting is configured.

15 Control Module	1	Run Time: 10.00 min	
515 Pumps Events 515 Pump (A) Initial Row Rate: 1.00 mL/min	Solvent Name:	Pressure Limits: Low: 0 bar High: 276 bar	
B Initial Row Rate: 0.00 mL/min	Solvent Name:	Pressure Limits: Low: 0 bar High: 414 bar	S
C Initial Row Rate: 0.00 mL/min	Solvent Name:	Pressure Limits: Low: 0 bar High: 414 bar	
	Config	OK Cancel	

- 9. Check the status of makeup pump.
 - a. Make sure it is at "Rem" (Remote) mode to be controlled by Masslynx software
 - b. The normal pressure should be around 200 psi.

If the pressure is < 0	D, call our staff to remove the air bubbles inside before injection.
RU	
10. Select Autosampler and sel	ect right loop 1000 μl.
Ceri	Waters 2767 Autosampler Injection Wash Auxiliary Fraction Mixing Stacked Injection
	Injection Parameters Loop Selection Left 20 µl ⊂ Right 1000 µl €

11. Determine the washing procedure of the autosampler.

- a. Wash after injection: suitable for sample that has long retention time.
- b. Wash after collection: suitable for short experiment.

	19 2 3 [k] 4				
Wate	rs 2767 Autosamp	ler			
	on Wash Auxiliary Fraction	Mixing Stacked Injec	tions	, ji	
Status	and second second				
	Wash Parameters				0
Inlet	Wash Alter:	C Injection	Collection		CX
inec	Wash Probe Vent	0.5255985			$\mathbf{\nabla}$
5	Wash Pump Facto				
utosampler	Number of Wash S		(* 2		
	Inject Port Wash 1				
Vaters 2998					

12. For the setting of PDA detector and QDa mass detector, please refer to Part D analytical analysis steps 12-17.

ii. Set up fraction collection method

13. Right click the "fraction file" in the sample list and the FractioLynx Method will be shown in the window as shown below.

			Fractionlyns Mathod - CPOS preparative_thrmplate.fbc File: View: Help	3
			Ornersi Twong Collection E3 E5+ APo1 APo1+ Analog PDA UV Trend Events Secondary Trigger	
e Is Empty			Factor Selector	
HS File 1	Fraction File March March March C	WaveLeigh A W	Factor Colector (b)	
TL preparative, lemplate D	OS_pepai. 227.1 282.1 371.2	0.000	Fraction CollectionDn	
05_preparative_wash	Browse_	0.000		
Association and a second SC	Edit	0.000	Nek Tox Prozeitive U	
	Cut Copy Paste	0.000 0.000 0.000 0.000	Value UHAs Horinari Factor Wath 3 The Boot	
	Add Insert	0.000	24	
	Clear Selected	0.000		
	Contornioe Display		Eper (w/ anu) (2.5	
	AutoSampler Bed Layout 2787 Vial Converter			

14. In the tab of "General",

a. Turn on the fraction collection. You can turn it off during trials before actually collecting fractions.

Analytical - Sliding 3-p	point calculation
Intensity of 3 rd data point must exceed the 1st data point by Leading Edge Gradient %	
Preparative - Sliding	5-point calculation
Average Intensity of last 3 data points must exceed the 1st data point by Leading Edge Gradient % MIT	
b. Peak narrower than set value will not be co	ollected.
General Timing Collection ES- ES+ APcI- APcI-	+ Analog PDA UV Timed Events Sec
Fraction Detection	
a. Fraction Collection:	On v
b. Peak Type:	Preparative ~
C. Minimum Fraction Width:	Value Units 3 Time (secs) ~
entrefor	

- 15. In the tab of "Timing",
 - a. Avoid collection of early eluted peak that may contain impurity
 - b. There is time delay between flow path to detector and flow path to collector. This delay depends on flow rate. Set according to the table below:

	Flow rate (mL/min)	Delay (secs	3)
	20	11	
	15	16	C
	10	21	
neral Timing Collect	a. Solvert Front Delay (a	ecs): 0	
Peak Timing			
	b. Spit/Collector Delayls	ecs): [21	
	MS/Analog Delay (sec	cs): 0	

- 16. In the tab of "Collection",
 - a. set the maximum fraction collected in one injection.
 - b. One fraction can be collected to more than one tube, so normally set b = 2a.
 - c. To prevent overfill of tube. Normally set as 90% for easy handling, i.e. 90% of a 15 mL tube = 10 mL will be filled.
 - d. Collection will terminate after set value. This will override "terminate peak" setting in "ES+" tab.

Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023

а	Max. Fracti	ions per Inje	ection:				10)	
b	• 🗹 Max. Tube	s per Injecti	ion:				20)	
	🗌 Finish Sam	ples after M	lax Frac	tions					
	Minimum Run Ti	me(mins) :							
С	Maximum Tube F	Fill (%):					90)	
d	Maximum Fractio	n Width:		Time (se	ecs)	~	30)	

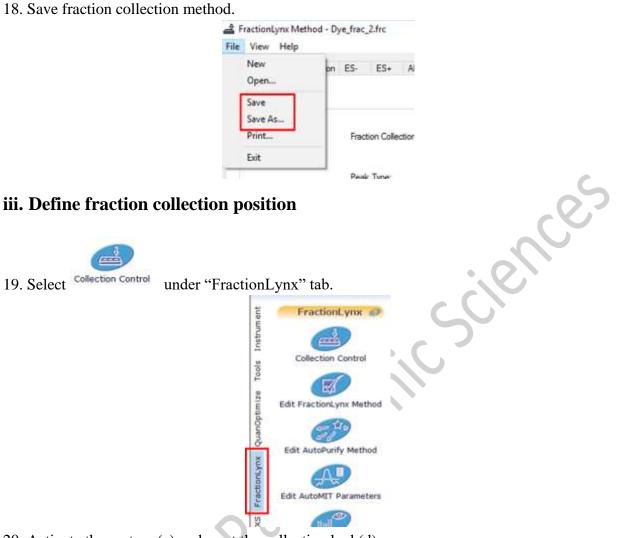
- 17. In the tab of "ES+",
 - a. 0, 1 (+H), 23 (+Na)
 - b. The minimum response of the target required for collection to occur. To avoid collection of undesired fractions, the MIT must be set to a value greater than the intensity of the background of the detector.

It can be determined by:

- Previous experience, e.g. QDa mass detector often have background of at least 1e5 level
- Run a trial
- Run a solvent blank
- Auto MIT (run a blank and allow software to calculate MIT)

General	Timing Collection ES- ES+	APcI- APcI+ Analog PD	A UV T	imed Events	Secondary Triggers
ES+ lor	Detection				
	a. ES+ Ion Adducts:		0,1,23		
	b. Mn. Intensity Three	hold (MIT):	4e5		
2	C. Max. Intensity Three	shold.	1.5e8		
		Type		Value	
5	C. Peak Stat	Use MIT Only	0	0	
	Terminate Peak:	Use MIT Only		14	

Centre for PanorOmic Sciences, LKS Faculty of Medicine, HKU Waters AutoPurification System 2023



20. Activate the system (c) and reset the collection bed (d).

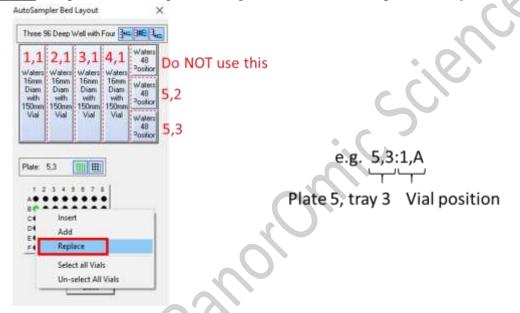
File New System Help C.	
😂 * 🗱 System 👌 Shirtcat 🍫 Actuals	Ready
System Cantrol • View • Fun • Reset Corrects	Waters 2767: Three 96 Deep Well with Four 18mm Rac
a. b. weiers 2767	50mm Vial Waters 16mm Diam with 150mm Vial Waters 16mm Diam with 150mm Vial
	e.

iv. Create sample list

21. In the sample list:



- a. <u>File Name</u>: sample name. Only include alphabets and "_". Maximum 20-30 characters, otherwise may generate errors.
- b. Inlet File: load your established Inlet method file.
- c. <u>Bottle</u>: the position of sample vial. Right click \rightarrow "AutoSampler Bed Layout"



- d. <u>Inject Volume</u>: maximum 1000 μ l for preparative run but recommend to start with 100 μ l to prevent contamination of the system.
- e. <u>MS File</u>: load your built MS method file.
- f. <u>Fraction File</u>: load your built fraction collection method file.
- g. <u>Mass A/B/C or Wavelength</u>: enter the molecular mass or wavelength of your targets.
- h. <u>Fraction trigger 1/2/x</u>: double click to select the corresponding triggers.
- i. <u>Fraction Start</u>: define starting collection tube position (optional).
- j. Other columns can be left blank.
- 22. Right click sample list to add more samples.
- 23. Select **b** to save your sample list.

A Before running your sample, check the following:

- The pressure of the pump is less than 2000 psi. If it is >2000 psi, call our staff. •
- The pressure of the pump is stable (fluctuating within +/-50 psi). •
- No leakage of solvent from the two extremes of column(s)
- The sample vials are in the right position as defined in your sample list.
- The fraction collection tubes are in the right position as defined in fraction collection method.

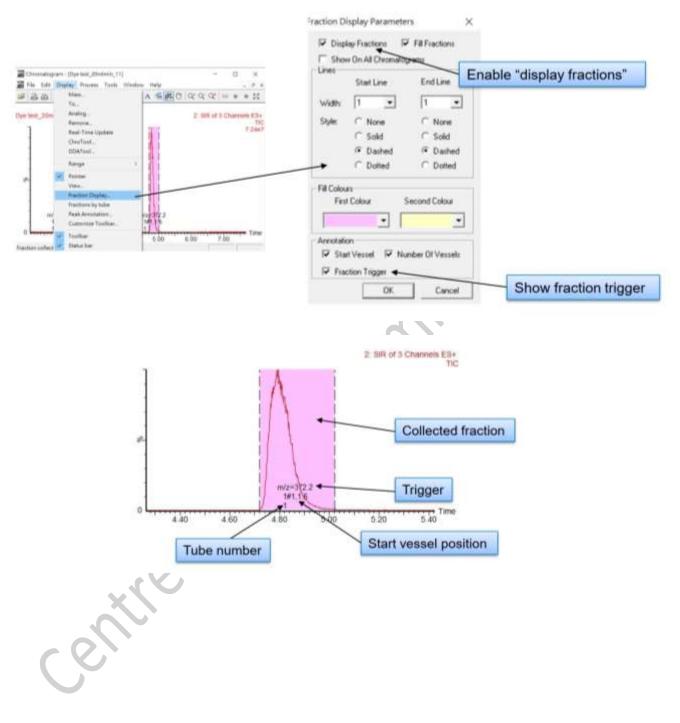
24. Highlight the samples to be run and select to start the run.

×refr

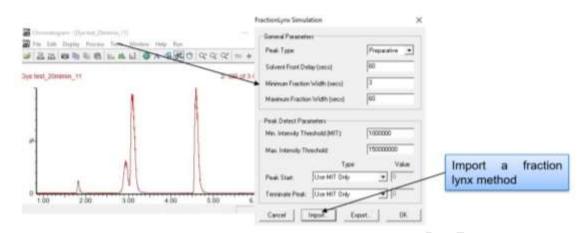
After experiment, remember to remove waste in the waste bottle.

V. View collection results

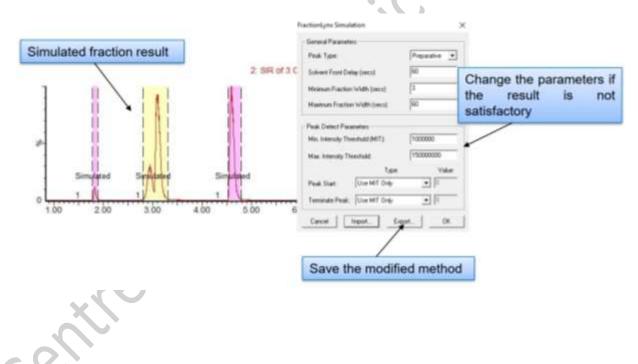
25. In the window of chromatogram, select "Display" → "Fraction Display" to enable Display fractions and show fraction trigger.



26. If you found the fraction result is not good enough or if you have run a trial and want to modify the fraction collection method, select "Process" → "FractionLynx Simulation". Import a fraction collection method for modification.



27. Change the parameters and the fraction result will be simulated in the chromatogram. Save the modified fraction collection method.



F. Washing and shutdown

1. Run two blanks (Methanol) with "CPOS_Wash" as inlet method and "CPOS_wash" as MS method (to monitor any contaminates left) to wash the column and system For example,

Spe	ctrum Chromatogram Mag	Edit - Samples -					
	File Name	Inlet File	Bottle	Inject Volume	Process	Parameter File	MS File
1	wash1	CPOS_wash	5,21,C	0.000			CPOS_wash_MS
2	wash2	CPOS_wash	5,2:1,C	0.000			CPOS_wash_MS

2. Enable shutdown after batch (all the samples waiting in queue). This will turn off the flow, PDA detector and Qda mass detector.

a. Click "Shutdown Disabled' at the bottom right-hand corner.

	File Edit View Control List Shutdown Log Image: Shutdown Auto Control Tasks Image: Shutdown Auto Control Tasks		H
	Batch Control	upLCOnly_ACE.acl	Browse
	Enable shutdown C:\MassLynx\ShutDown\Shut Shutdown if queue is in pause	DownESI_ACE.acl	Browse
	Shutdown Time Shutdown Time I.00	nutdown On Error Configure error shut	down
	Optimization E-mail on Error Shutdown		
	<u> </u>		
Select Make s	to save the setting. sure "Only Batch Shutdown Enabled" is sho	own.	U.

- 3. Keep "MassLynx" software open, otherwise QDa mass detector will be disconnected.
 - 4. Keep the PC and other parts of instrument on.

G. Data transfer

- NO personal USB thumb drive to the PC connecting to Waters autopurification system
- Use CPOS USB thumb drive to transfer data to data transfer stations shown below
- On data transfer stations, you can copy data with your own USB thumb drive or through uploading to Imaging and Flow Cytometry Core Server

Data Transfer Station for Waters Autopurification System

(WITH Masslynx software for analysis)



Located at the end of the lane of Waters autopurification system

<u>Data Transfer Station</u> (WITHOUT Masslynx software for analysis)









Located at one lane behind Waters autopurification system