# Waters

### Introduction

Mass-directed purification has proved a useful tool in streamlining the purification process. For mass-directed purification to work, the target has to ionize, which can sometimes be a challenge. Often both ESI and APCI are required for a given sample or sample set. All previous approaches addressing this issue reduced the efficiency and throughput of the purification process. Some approaches included:

- 1. Running with a single ionization mode.
- Samples are lost. Could claim they were never there.

#### 2. Run in both modes

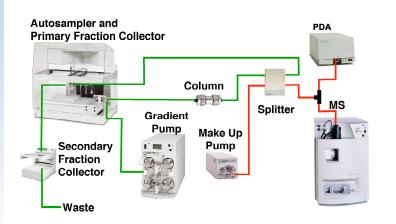
- Samples and fractions divided into 2 subsets, making sample tracking and reformatting difficult.
- 3. Collect by a non-specific detector (UV or ELSD)
- Many samples to be reanalyzed and still require both modes of ionization, shifting the problem downstream.

A superior approach is to use the Waters® ESCi<sup>™</sup> Multi-Mode ionization source. This is capable of high-speed switching between ionization modes enabling ESI and APCI and positive/negative switching to occur throughout the analysis.

Using ESCi with FractionLynx<sup>™</sup> allows mass-directed purification to be driven from ESI<sup>+</sup>, ESI<sup>+</sup>, APCI<sup>+</sup> or APCI<sup>-</sup> data in a given run. This poster shows the purification of a sample set using ESCi for massdirected fractionation and demonstrates the effectiveness and efficiency that can be achieved utilizing this unique interface.

#### Equipment

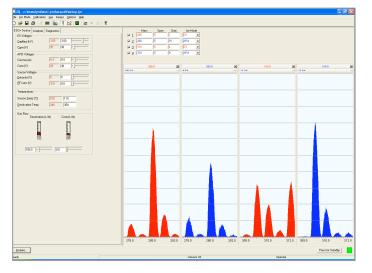
Waters Mass-Directed Purification System: 2525 Binary Gradient Module, 2767 Sample Manager, Column FluidicsOrganizer, 2996 Photodiode Array Detector, ZQ<sup>™</sup> Mass Detector with ESCi Multi-Mode Ionization Source controlled by MassLynx<sup>™</sup> Version 4.0 with FractionLynx, XTerra® C<sub>18</sub> 5 µm Column 19 x 50 mm.



Flow diagram of the multi-mode ionization mass-directed purification system.

#### **Experimental Conditions**

- Samples were prepared by mixing various drug like test compounds with about 20 mg total compound loaded per injection.
- 20 mL/min linear binary gradient from 2.5 70% water: acetonitrile with 0.1% formic acid over 7.5 minutes.
- 1:1000 split with a 1 mL/min makeup flow of 50% water: acetonitrile with 0.1% formic acid. The makeup flow is split: 80% to the UV, 20% the MS.
- •MS conditions—Tune Page



MassLynx Tune Page with multi-mode ionization. Parameters for ESI<sup>+</sup>/- and APCI<sup>+</sup>/- are set independently.

#### • Acquisition parameters—MS method

S Scan						
Total Run Time; 10.00 ↔						
Time						

MassLynx MS Experiment Setup window. Ionization modes can be chosen in various combinations and durations.

### **Entering Sample Information**

- All sample information is entered into the sample list. This includes the target mass(es) and the fraction collection triggers.
- When doing mass-directed purification with Multi-Mode ionization, all scans defined in the MS method are monitored for each mass entered.
- For example, with a target mass of 134, a fraction will be collected if mass 135 is seen in the ES<sup>+</sup> or AP<sup>+</sup> scan or mass 133 in the ES<sup>-</sup> or AP<sup>-</sup> scan

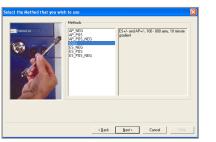


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	File Name	Inlet File	Bottle	Inject Volume	MS File	Fraction File	Mass A	Mass B	Mass C	Fraction Trigg.	Fraction Trigg.	Fraction Trigg.	Process	Parameter Fi
1	ESCi_Prep_1	Generic1	5,21,A	1000	esci	ESCi	134.0	139.1	236.3	Mass A	Mass B	Mass C	AutoPurity	ESCillp
2	ESCLPrep_2	Genetic1	5,2:1,8	1000	esci	ESCI	371.4	308.1	259.1	Mass A	Mass B	Mass C	AutoPutity	ESCLIP
3	ESCi_Prep_3	Genetic1	5,2:1,C	1000	esci	ESCI	171.1	279.3	210.3	Mass A	Mass B	Mass C	AutoPurity	ESCillp
4	ESCi_Prep_4	Generic1	5,21,D	1000	erci	ESCI	194.2	134.1	264.3	Mare A	Matt B	Mass C	AutoPurity	ESCillp
5	ESCi_Prep_5	Genetic1	5,2:2,A	1000	esci	ESCi	127.1	260.2	375.3	Mass A	Mass B	Mass C	AutoPutity	ESCifip
6	ESCi_Prep_6	Genetic1	5.2.2,B	1000	esci	ESCI	151.1	162.2	277.4	Mass A	Mass B	Mass C	AutoPurity	ESCifip
7	ESCi_Prep_7	Generic1	5,2.2,C	1000	esci	ESCI	148.1	134.0	151.2	Mass A	Mass B	Mass C	AutoPurity	ESCillp
8	ESCi_Prep_8	Genetic1	5,2:2,D	1000	esci	ESCi	196.2	371.4	357.3	Mass A	Mass B	Mass C	AutoPurity	ESCifip
9	ESCi_Prep_9	Genetic1	5,23,A	1000	esci	ESCI	127.0	148.1	268.2	Mass A	Mass B	Mass C	AutoPurity	ESCifip
10	ESCi_Prep_10	Genetic1	5,2:3,B	1000	esci	ESCi	268.3	282.3	357.3	Mass A	Mass B	Mass C	AutoPurity	ESCifip
11	ESCI_Prep_11	Genetic 1	5,2:3,C	1000	esci	ESCI	270.3	268.3	371.4	Mass A	Mass B	Mass C	AutoPutty	ESCLIP
12	ESCi_Prep_12	Genetic1	5.2.3.D	1000	esci	ESCI	134.1	294.8	409.2	Mass A	Mass B	Mass C	AutoPurity	ESCifip
13	ESCLPrep_13	Generic1	5,24,A	1000	erci	ESCI	229.2	295.8	371.4	Marr A	Mate B	Mase C	AutoPurity	ESCLIp
14	ESCi_Prep_14	Genetic1	5,2:4,B	1000	esci	ESCI	151.1	278.3	590.3	Mass A	Mass B	Mass C	AutoPurity	ESCifip
15	ESCi Prep 15	Genetic1	5.24.C	1000	esci	ESCI	277.3	279.3	518.3	Mass A	Mass B	Mass C	AutoPurity	ESCifip
16	ESCi_Prep_16	Generic1	5,2.4,D	1000	esci	ESCI	171.2	194.2	308.4	Mass A	Mass B	Mass C	AutoPurity	ESCIIIp
17	ESCi Prep 17	Genetic1	525A	1000	esci	ESCI	294.2	409.2	421.1	Mass A	Mass B	Mass C	AutoPurify	ESCifip
18	ESCi_Prep_18	Genetic1	5,25,B	1000	esci	ESCI	210.3	171.1	357.4	Mass A	Mass B	Mass C	AutoPurity	ESCifip
19	ESCi_Prep_19	Genetic1	5,25,C	1000	esci	ESCi	278.3	251.2	134.0	Mass A	Mass B	Mass C	AutoPurity	ESCifip
20	ESCI_Prep_20	Genetic 1	5,2:5,D	1000	esci	ESCI	155.1	260.3	375.3	Mass A	Mass B	Mass C	AutoPutty	ESCLIP
21	ESCi_Prep_21	Genetic1	5.26A	1000	esci	ESCI	610.3	279.2	269.2	Mass A	Mass B	Mass C	AutoPurity	ESCillp
22	ESCI_Prep_22	Generic1	5,2.6,B	1000	erci	ESCI	371.4	162.2	216.3	Mare A	Mate B	Masy C	AutoPurity	ESCLIP
23	ESCi_Prep_23	Generic1	5.2.6.C	1000	esci	ESCi	199.0	134.0	357.4	Mass A	Mass B	Mass C	AutoPurity	ESCIIIp
24	ESCi Prep 24	Genetic1	5.26.D	1000	esci	ESCI	1481	268.2	278.2	Mats A	Mass B	Mass C	AutoPurity	ESCillo

MassLynx Sample List of 24 samples with 3 mass targets per sample.

# **Open Access Login**

• An alternative for entering the sample information is to use Open Access Login.

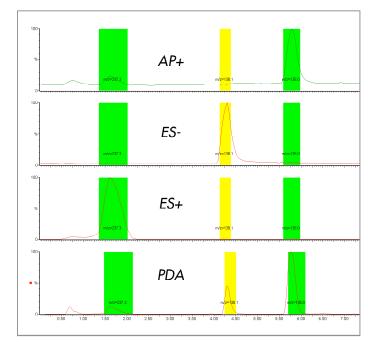


The OA Login screen, where the desired OA method is selected. The OA method contains the specific LC, MS and Fraction Collection methods.

• The ESCi Multi-Mode ionization source is ideal for open access because it is not necessary to change the source for any mode selected.

# **Sample Purification**

• The desire for this sample was to collect the 3 major peaks. However, the targets are only seen in different ionization modes



Fractionation of a 3 component mixture. One peak is seen in ES<sup>+</sup>, one in ES<sup>-</sup>, and one in AP<sup>+</sup>.

- All 3 fractions were successfully collected on a single injection.
- Sample does not have to be divided to be purified. No sample lost.
- No need to fractionate with a generic detector, then reanalyze all the fractions both modes. Throughput and efficiency is increased.

# Library Purification

- The goal of this example was to purify a library of 24 samples, each with 3 targets for a total of 72 targets.
- Prior analytical data found targets in all ionization modes.

Target Breakdo	own	Sample Breakdown				
Number of Targets	Ionization Mode	Number of Targets	Ionization Mode ES+			
36	ES⁺	23				
12	2 AP* 12		AP⁺			
22	ES <sup>.</sup>	20	ES <sup>.</sup>			
1	AP <sup>.</sup>	1	AP <sup>.</sup>			

\*12 Samples contained both ES and AP targets

- Sample were purified using mass-directed purification with the ESCi Multi-Mode Ionization source
- The FractionLynx Browser graphically displays the purification results.



The FractionLynx Browser displays the trigger and ionization mode for each collected fraction, along with other useful information about the purification.

- All 72 targets collected into 1 rack in a single run
- No targets are lost with ESCi
- If only ES<sup>+</sup>/- used 17% of the targets would be lost
- Libraries with varying ionizing targets are easily purified used ESCi
- Using the ESCi Multi-Mode ionization source for mass-directed purification reduces the risk of not seeing a target. This added security increases the sample throughput and efficiency of the purification process.

Paul M Lefebvre, Robert Plumb, Warren B Potts III, Ronan Cleary

# Other Benefits of ESCi

- No loss in sensitivity with mode switching<sup>1</sup>
- No data cross-talk between acquisition channels<sup>1</sup>
- LC flow rate is constant for both modes
- No need to change the plumbing or split ratio when changing ionization modes
- Faster interscan delay
- Decreased from 0.3 with Pos/Neg switching to 0.1 seconds

# Conclusion

- Waters ESCi Multi-Mode Ionization Source is capable of high-speed switching between ionization modes enabling ESI and APCI and positive/negative switching to occur throughout the analysis.
- ESCi with FractionLynx<sup>™</sup> allows mass-directed purification to be driven from ESI<sup>+</sup>, ESI<sup>+</sup>, APCI<sup>+</sup> or APCI<sup>+</sup> data in a given run.
- The summary of benefits include:
  - Reduces the number of missed targets.
  - Eliminates the need to divide the samples or sample sets.
  - Keeps all fractions together for simple tracking and handling.
  - Increases overall throughput and efficiency of the purification process.
- No source change required, ideal for open access.

#### Acknowledgements

- Mike Baloah
- Darcy Shave
- Jo-Ann Jablonski
- Tom Wheat

# References

- 1. Richard T. Gallagher, Michael P. Balogh, Paul Davey, Mike R. Jackson, Ian Sinclair, and Lisa J. Southern, Anal. Chem., 75 (4), 973 -977, 2003
- 2. Source Design and the Utility of Multimode Ionization, Michael P. Balogh, LC/GC North America, Vol 21 No 10, 984 - 991, October 2003LC/GC Article

ESCi Multi-Mode Ionization patent (pendina) Serial No. PCT/US03/16892 ©2004 Waters Corporation 720000944EN MB-PDF