Waters

MASS-DIRECTED PURIFICATION USING MULTI-MODE IONIZATION

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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:

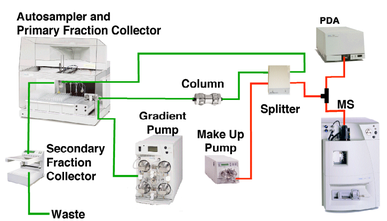
1. Running with a single ionization mode.
   - Samples are lost. Could claim they were never there.
2. Run in both modes
   - Samples and fractions divided into 2 subsets, making sample tracking and reformatting difficult.
3. Collect by a non-specific detector (UV or ELSD)
   - Many samples to be reanalyzed and still require both modes of ionization, shifting the problem downstream.

A superior approach is to use the Waters® ESCi™ 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™ allows mass-directed purification to be driven from ESI⁺, ESI, APCI⁺ or APCI data in a given run. This poster shows the purification of a sample set using ESCi for mass-directed fractionation and demonstrates the effectiveness and efficiency that can be achieved utilizing this unique interface.

Equipment

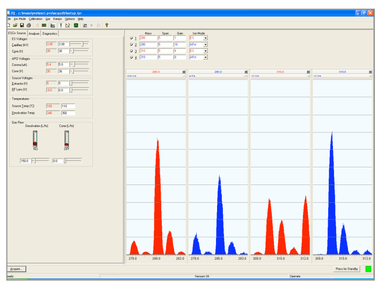
Waters Mass-Directed Purification System: 2525 Binary Gradient Module, 2767 Sample Manager, Column FluidicsOrganizer, 2996 Photodiode Array Detector, ZQ™ Mass Detector with ESCi Multi-Mode Ionization Source controlled by MassLynx™ Version 4.0 with FractionLynx, XTerra® C_{18} 5 µm Column 19 x 50 mm.



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

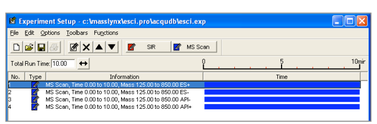
Experimental Conditions

- 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⁺/- and APCI⁺/- are set independently.

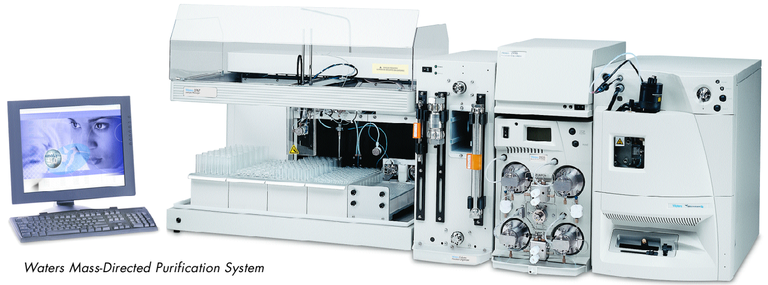
- Acquisition parameters—MS method



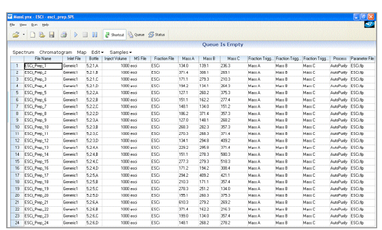
MassLynx MS Experiment Setup window. Ionization modes can be chosen in various combinations and durations.

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⁺ or AP⁺ scan or mass 133 in the ES⁻ or AP⁻ scan.



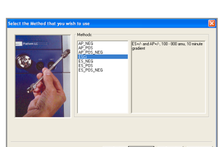
Waters Mass-Directed Purification System



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.

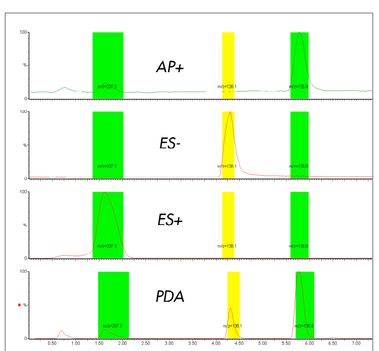


The OA Login screen, where the desired OA method is selected. The OA method contains the specific LC, MS and Fraction Collection methods.

- 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⁺, one in ES⁻, and one in AP⁺.

- 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 Breakdown

Number of Targets	Ionization Mode
36	ES⁻
12	AP⁻
22	ES⁻
1	AP⁻

Sample Breakdown

Number of Targets	Ionization Mode
23	ES⁻
12	AP⁻
20	ES⁻
1	AP⁻

**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⁺/- 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.

Other Benefits of ESCi

- No loss in sensitivity with mode switching[1]
- No data cross-talk between acquisition channels[1]
- 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™ allows mass-directed purification to be driven from ESI⁺, ESI, APCI⁺ or APCI⁻ 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 Balogh
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- 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 [pending] Serial No. PCT/US03/16892