PosterREPRINT

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MILLIGRAM SCALE PURIFICATION INTO A SINGLE WELL OF A 96-WELL PLATE

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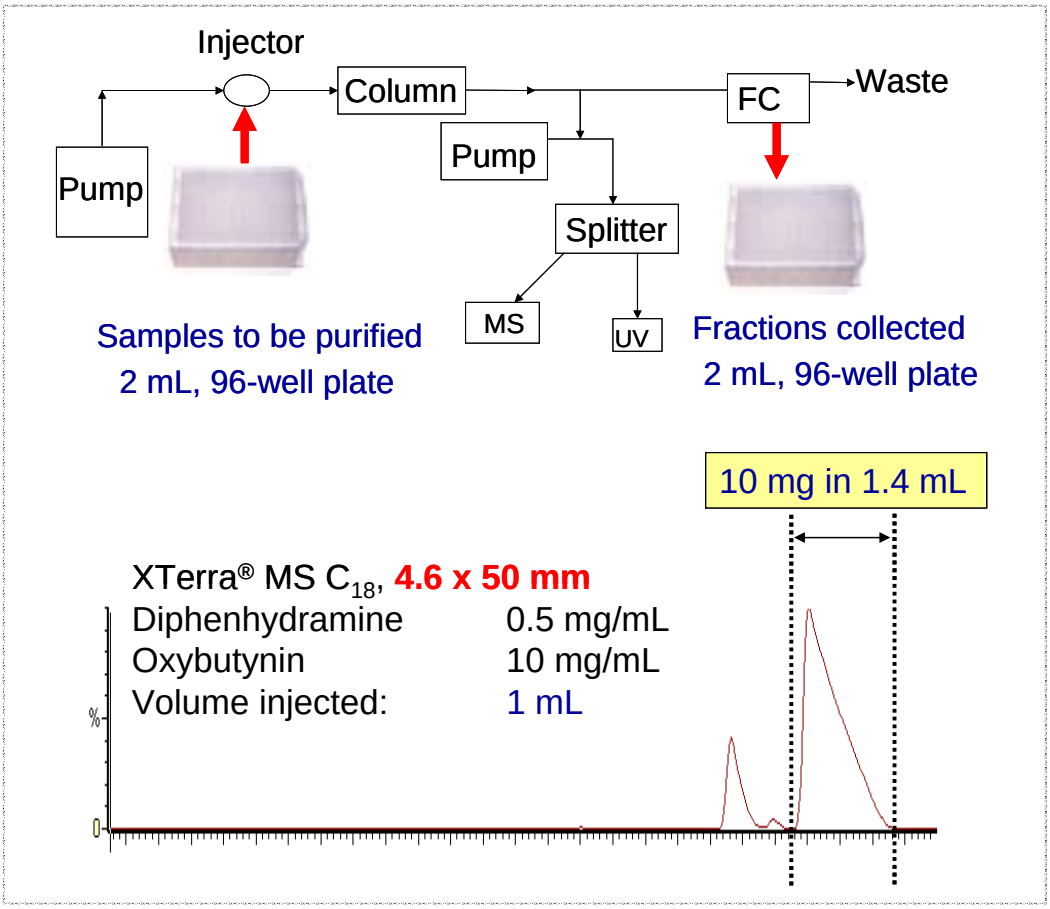
OVERVIEW

In order to minimize discovery timelines, reduction in sample handling is key. There are cases where samples produced by combinatorial synthesis contain only a few milligrams of the targeted compound and many of the libraries developed are handled for purification in a 96- well format. Ideally, samples could be loaded from a 96-well plate and the pure fraction collected onto a well of another 96-well plate. However, this requires a small fraction volume for collection, in fact, less than 2 mL. We have been able to demonstrate that it is indeed possible to carry out such purifications employing an analytical size column injecting over 10 mg of a given mixture and collecting the pure compound in one fraction. These results illustrate that running preparative chromatography in a smaller column results in lower column costs, lower flow rates, lower pressures, lower fraction volumes, less solvent to dispose and reduced sample handling.

INTRODUCTION

Combinatorial chemistry samples are often synthesized in 96-well plates. Chemists are looking for the fastest and easiest means possible to purify these samples. If samples on the order of 1 to 10 mg can be purified by reversed-phase chromatography utilizing an analytical column (i.e. 4.6 x 50 mm), fractions can be collected into a single well in a plate-to-plate mapping set-up.



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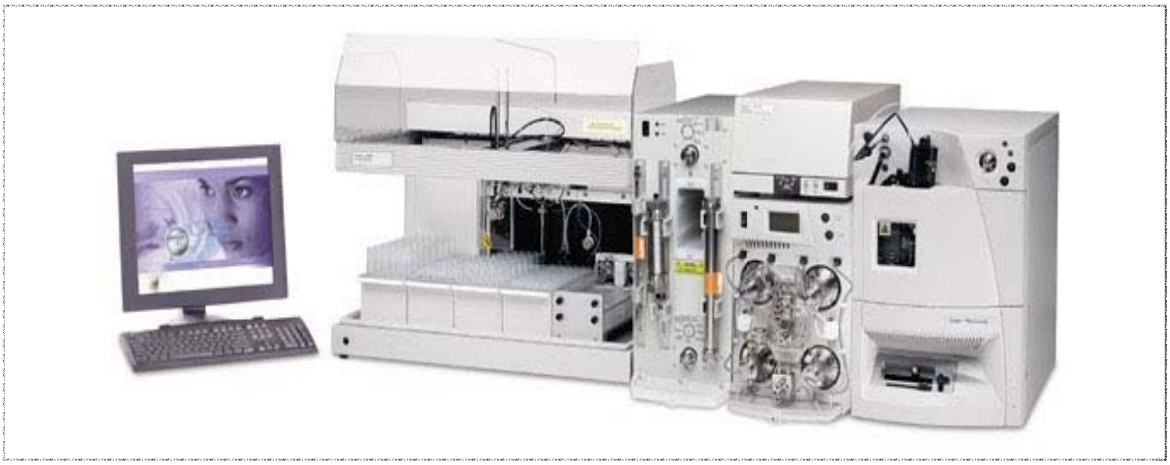
METHODS

FractionLynx™ AutoPurification™ System:
Waters® 2767 Sample Manager
Waters® 2525 Binary Gradient Module
Waters® 2996 PDA Detector
Waters® Micromass® ZQ™ Mass Spectrometer
Waters® Active Flow Splitter, split factor of 22
Waters® 515 HPLC Pump



CHROMATOGRAPHIC CONDITIONS:

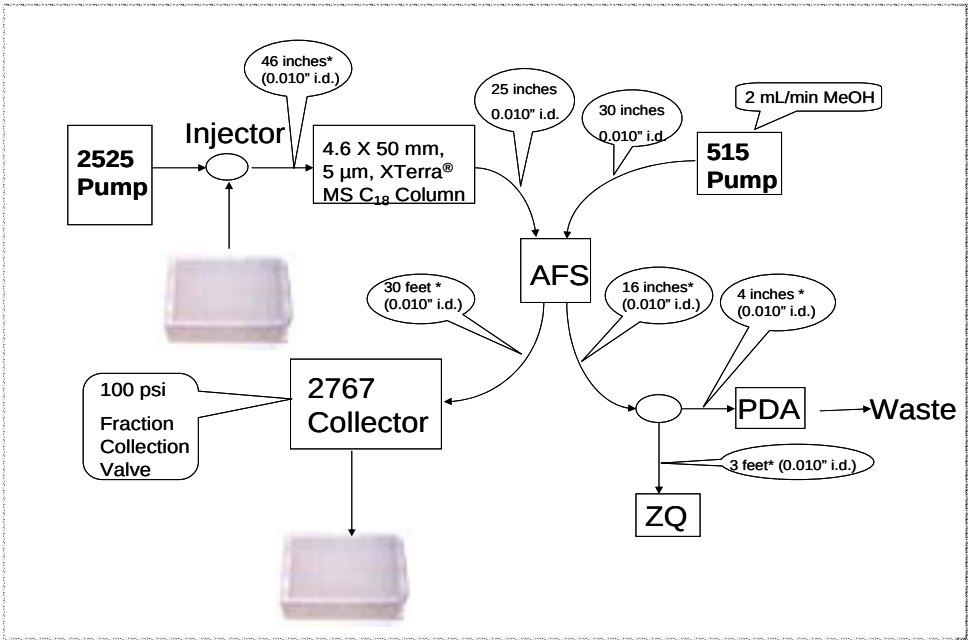
Column:	XTerra® MS C_{18}, 4.6 X 50 mm, 5 µm		
Part Number:	186000482		
Mobile Phase A:	Water:100 mM NH_4HCO_3, pH 10 (90:10)		
Mobile Phase B:	MeOH:100 mM NH_4HCO_3, pH 10 (90:10)		
Flow Rate:	1.8 mL/min		
Gradient:	Time(min)	%A	%B
	0.00	95	5
	1.00	95	5
	6.00	5	95
	6.01	0	100
	7.00	0	100
	7.01	95	5
	12.00	95	5

MASS SPECTROMETER CONDITIONS (ES+):

Capillary (kV)	3.0
Cone (V)	30
Extractor (V)	3
RF Lens (V)	0.3
Source Temperature (°C)	100
Desolvation Temperature (°C)	350
Cone Gas Flow (L/Hr)	150
Desolvation Gas Flow (L/Hr)	250
LM Resolution	15
HM Resolution	15
Ion Energy	0.3
Multiplier (V)	500

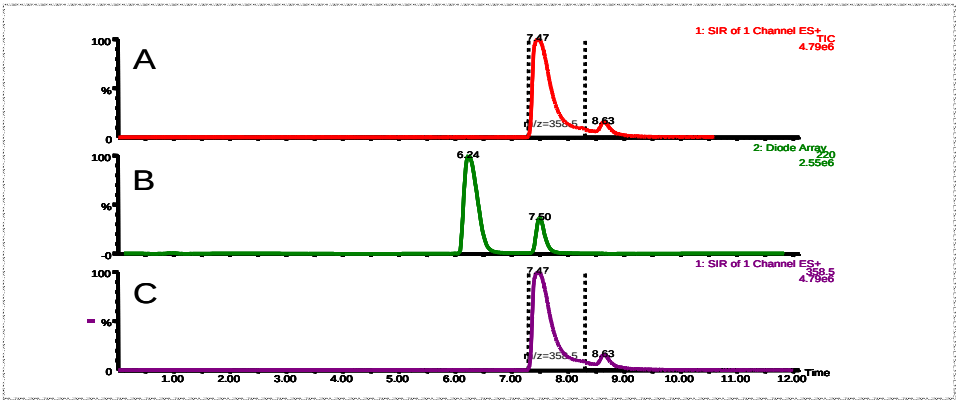
RESULTS AND DISCUSSION

Plate-to-Plate Mapping System Configuration. * indicates a crucial length and inner diameter of tubing. We incorporated the Waters Active Flow Splitter (AFS) and a make-up pump (for dilution) for two reasons. One, we needed to prevent the MS from becoming overloaded with analyte. Two, we wanted to have a much of the flow as possible going to the fraction collector for the highest recoveries.

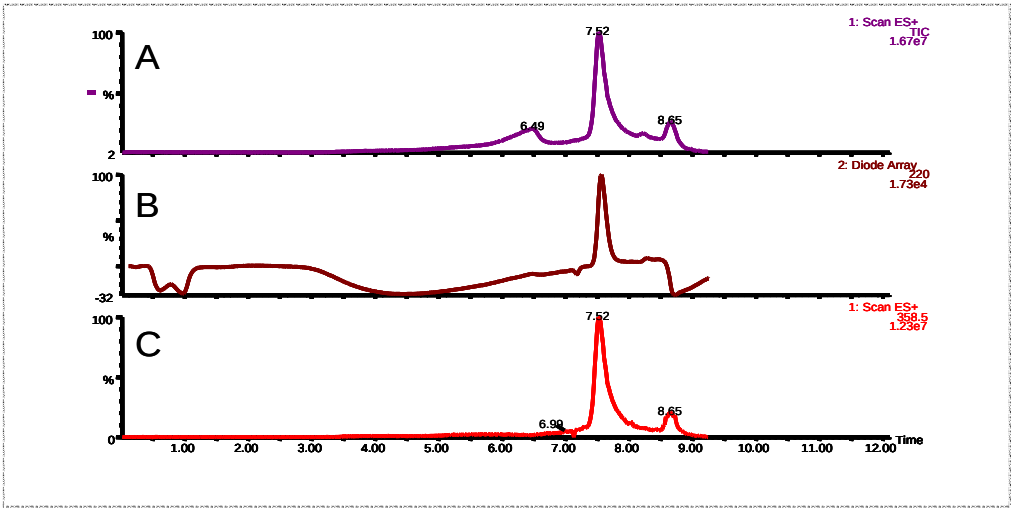


PROOF OF CONCEPT

We made a sample mixture of doxylamine and oxybutynin in DMSO, 2 mg/mL each. The MS was set up to trigger collection at m/z of 358.5, oxybutynin. We made an 800 uL injection of the sample mixture. A) Total ion chromatogram of one SIR channel, m/z 358.5. B) UV at 254 nm. C) Extracted chromatogram of m/z 358.5. Dashed lines indicate fraction collection.

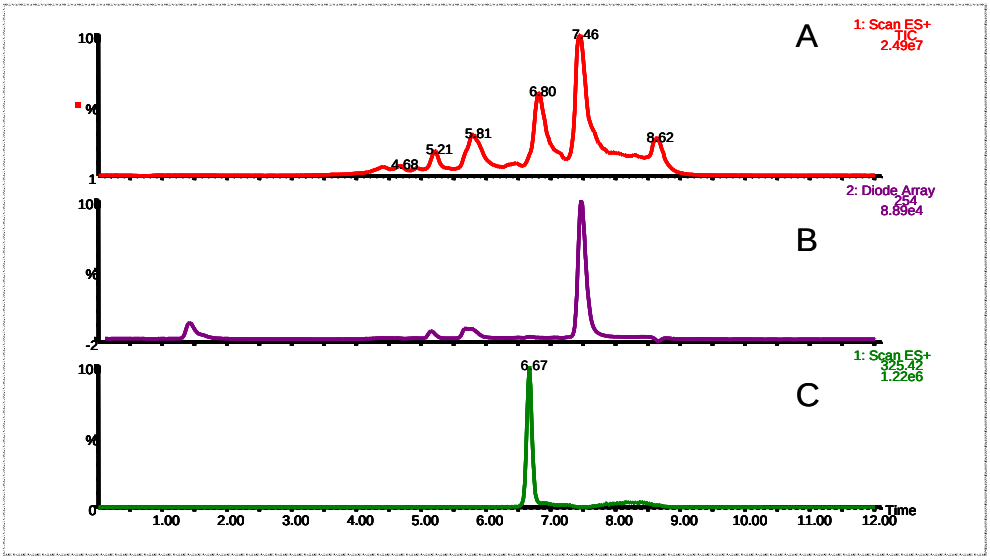


We then made a 20 µL injection of the collected fraction. A) Total ion chromatogram. B) UV at 220 nm. C) Extracted chromatogram of m/z 358.5. We successfully collected 1.5 mg into one 2-mL well of the plate.

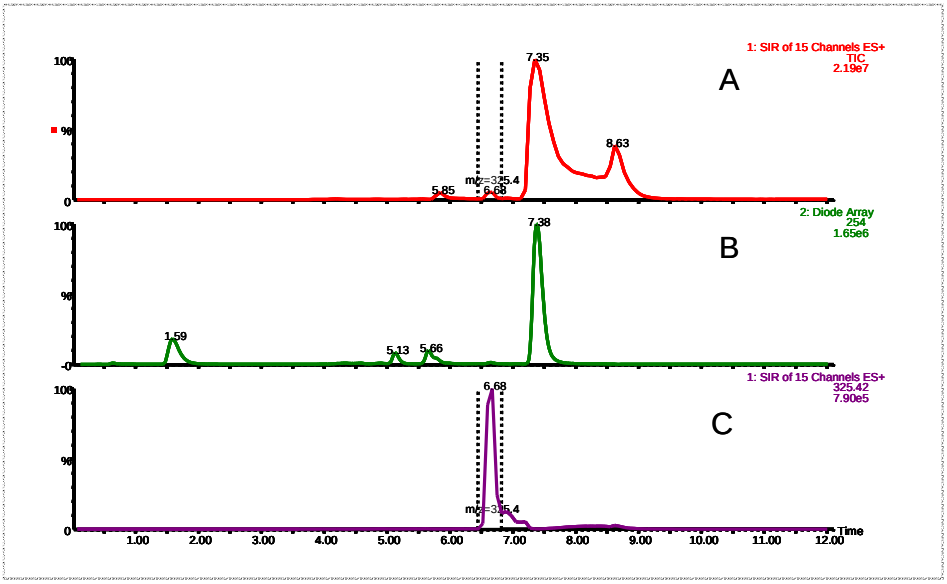


REAL WORLD SAMPLES

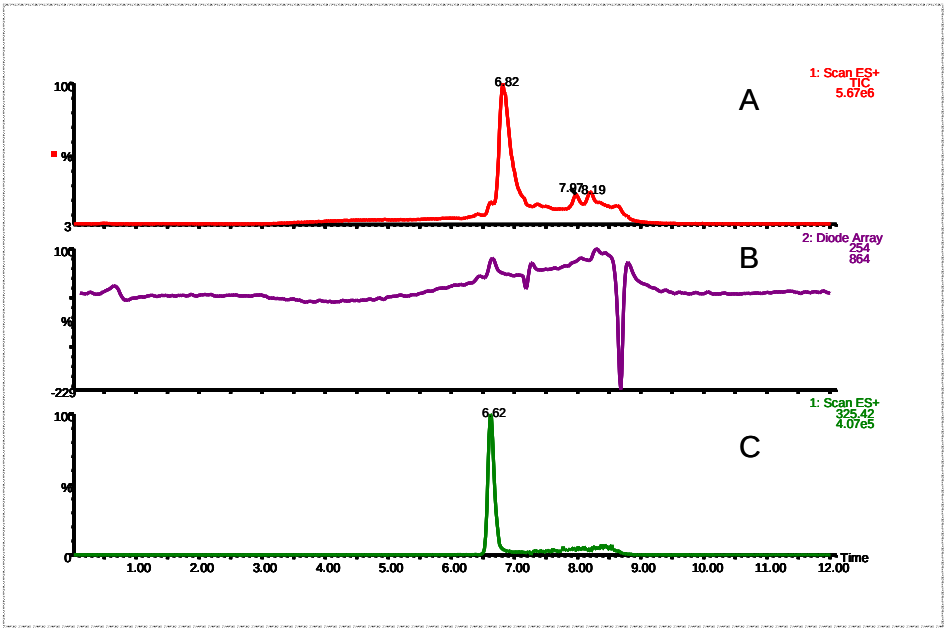
After we determined that the set-up was working, we tested the system with samples in a 96-well plate obtained from a company generating combinatorial chemistry libraries. First, we made a 20 µL injection of the undiluted sample in the well to determine whether or not the mass we were interested in was present in the sample. A) Total ion chromatogram. B) UV at 254 nm. C) Extracted chromatogram of m/z 325.4, corresponding to the analyte of interest. Note that the analyte of interest is not detected by the PDA.



We set up the mass spectrometer to trigger collection using m/z 325.4. We then made an 800 µL injection of the sample. A) Total ion chromatogram of 15 SIR channels. B) UV at 254 nm. C) Extracted chromatogram of m/z 325.4. Dashed lines indicated fraction collected.



We then made a 20 µL injection of the collected fraction. A) Total ion chromatogram. B) UV at 254 nm. C) Extracted chromatogram of m/z 325.4.



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CONCLUSIONS

We have developed a system to purify small quantities (up to 10 mg) of analytes from one well of a 96-well plate, collected into a single well on another 96-well plate. Using the Waters Active Flow Splitter, we can successfully split 1/4000 of the HPLC flow and dilute it with MeOH before sending it to the mass spectrometer. This means that 3999/4000 (99.975%) of the HPLC flow goes to the Fraction Collector. By using XTerra® MS C_{18} columns at high pH, we can maximize the loading and peak shape of basic analytes so the entire sample can be collected in less than 2 mL.

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