High Throughput Mass-Directed Purification of Drug Discovery Compounds

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Introduction

With combinatorial chemistry laboratories setting production targets in excess of 100,000 compounds per year, it is likely that over 300,000 samples will need to be created annually due to high compound attrition rates. These libraries must be screened and purified before use by pharmaceutical companies. To support this activity, it is not unusual for contract labs to have single contracts requiring the purification of 100,000 compounds per year.

The ability to handle the productivity demands created by these libraries requires the use of purification systems with ultra-high throughput and low cost per sample. It has been shown that a four channel LC/MS purification system can reduce the time it takes to process a 16 plate combinatorial library down to 11 days, compared to 26 days for a single channel system. To meet this purification challenge, the Waters® Purification Factory™ was used to demonstrate the purification of almost 4000 samples in just 10 days (4 microtitre plates/day). Based on well-established technologies, including the Waters® Micromass® ZQ™ Mass Spectrometer, MUX-technology™, XTerra® Prep Columns and FractionLynx™ Application Manager, the Purification Factory provides automated mass-directed purification of four samples simultaneously. The multiplexing of sample streams into a single mass spectrometer provides a space and cost effective solution to these high throughput laboratories

Multiplexed Purification System

Waters® Purification Factory

Components

Four Waters 2525 Binary Gradient Modules, a 2777 Sample Manager with a 4 Injection Valve Modules, a 2488 Multi-channel UV/Vis Detector, a ZQ™ Mass Spectrometer with MUX-technology, 4 Waters Fraction Collector III's, a 515 Makeup Pump, and 4 LC Packings 1:1000 Splitters.

Purification Method

4 Waters® XTerra™ Prep MS C18, 5 µm, 19 x 50 mm columns
20 mL/min total flow water: acetonitrile: 0.1% formic acid gradient:
0-1 minute 5% B, 1-8 minutes 5-95% B, 8-9 minutes 95% B, 9-9.1 minutes 95-5% B, 10 minutes end

MS: ESI+, Capillary = 3.0 kV, Cone =20 V, Cone Gas = 100 L/hr, Desolvation Gas = 600 L/hr, Source Temp. = 120°C, Desolvation Temp. = 400°C, Scan Time = 0.5 sec, Interspray Delay = 0.1 sec

Sample Preparation

A mixture of sulfathiozole, ketoprofen and tylosin tartrate, 20 mg/ml of each component in DMSO was prepared. 500 uL of this mixture was repeatedly injected and the tylosin tartrate was collected. Four 96 well microtitre plates (MTP) of the sample mixture were purified every day for ten days

Four injections of 500 uL were performed simultaneously, one on each channel of the MUX system. With an inject-to-inject cycle time of approximately 11.5 minutes, it took only 18.5 hours to purify all 384 samples. Fractions were collected into 18 x 150 mm test tubes using the "1 for 1" collection mode. These fractions were then dried down on a Genevac R-4 (Genevac Inc, Valley Cottage, NY). Prior to fraction collection, representative subsamples of the tubes (10 per channel per day) were weighed.

A total of 400 dry samples (10 per channel per day) were weighed to determine recovery and then reconstituted in 1 mL of DMSO and transferred to 96 well MTP. The reconstituted samples were analyzed on an Alliance® HT HPLC System with a Quattro micro™ API tandem quadrupole mass spectrometer and a 2996 Photodiode Array Detector. The chromatographic method used the same gradient and profile as the purification but with a total flow of 1.2 ml/min on a XTerra MS C18 5 um, 4.6 x 50 mm column and a 100 uL injection volume.

System Throughput

Goal: *Increase throughput of the purification process while maintaining acceptable sample recovery and purity.*

- Gradient of 11.5 minutes required to meet the 85% purity requirement[2,3]
- 96 Samples x 11.5 minutes = 18.5 hours for purification
- 96 Sample x 4 simultaneous purifications = 384 samples in 18.5 hours
- 10 Days = 3840 samples purified @ 18.5 hours / day
- Over 5000 samples could have been purified in 10 days running at full capacity

This level of throughput requires an extremely robust and rugged system.

System Configuration

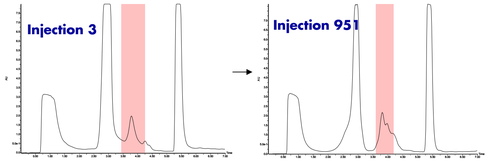
- The benefits of 4 preparative gradient pumps

1. Consistent flow across each column
   - The problem with the alternative approach of splitting the flow of 1 pump 4 ways is that any change in backpressure across any stream causes the flow to change on all the streams.

2. Independent gradient for each stream
   - Increases the number of different compounds and associated chromatography that can be analyzed
   - Allows for shallower gradients that focus in on the peak of interest, decreasing gradient run time. This capability can be automated using the AutoPurify capabilites of FractionLynx.[4]

Column Stability

- The Optimized Bed Density design of the Waters XTerra Prep columns allowed 30 mg of material to be loaded onto each column 960 times with little change in peak shape.

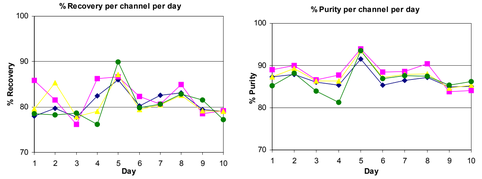


UV traces for the 3rd and 951st injection on the same column.

- Even with nearly 1000 injections per column, the columns showed acceptable performance and could be used for additional studies.[5]

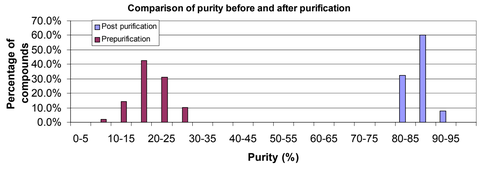
Purification Results

Compound purity was determined using percent peak area of the UV chromatogram. By multiplying total recovery by purity, we were able to determine how much of the target compound was collected.



Average recovery and recovery of tylosin tartrate fractions per channel per day. Each line represents an individual channel.

- Average purity was determined to be 87.3% with a standard deviation of 1.2%.

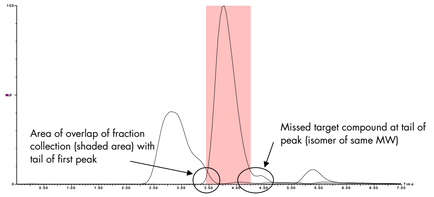


Purities of representative fractions before and after purification

- Before purification, all the samples were less than 30% pure. After purification, all the samples were over 80% pure and two-thirds were over 85% pure.

Balancing Throughput, Recovery and Purity

- The chromatography was optimized primarily for throughput and secondarily for purity and recovery. Therefore a compromise was made in choosing fraction collection parameters that balanced purity and recovery

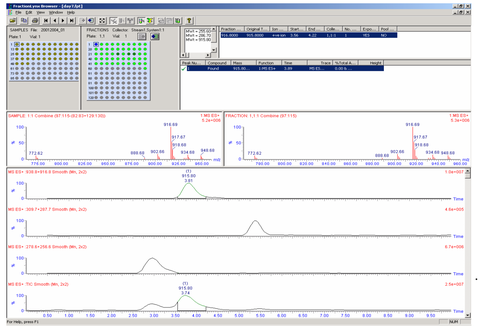


Overlaid extracted ion chromatograms of the three compounds present in mixture.

The threshold was set such that the tail of the first peak was collected and the tail of the target peak was not. Increasing the peak detection threshold would allow for the collection of purer fractions but at the cost of sample recovery. Lowering the peak detection threshold would allow for the collection of more sample but at the cost of purity.

Data Management

Being physically able to purify a large number of samples is only part of the equation for successful high throughput. Appropriate data management tools are required to process, report, and archive the necessary information. The FractionLynx Application Manager, used in conjunction with MassLynx, provides graphical presentation of the purification results for simplified sample and fraction tracking and viewing of all associated data. Furthermore, the browser is interactive and can automate the steps through the process.



The FractionLynx Browser displaying the sample and fraction plates, along with the appropriate information associated to the collected fraction

Additional Data Management Tools

- Waters NuGenesis® SDMS—Scientific Data Management System—is a self-generating electronic repository that stores and manages all scientific information. It utilizes "file and print capture" technology that consolidates and manages the generated data. NuGenesis SDMS can also exchange data with e-lab notebooks, LIMS, EDMS or other common systems.

- Waters eLab Notebook™ software allows researchers to capture, process, and record data of all types in a completely digital environment. It can be adapted to the existing workflow to increase the productivity.

  - For example, the synthetic chemist who requires the purification can submit an electronic request for services with all the necessary information linked. The results of the purification can then be inserted directly into the experimental record electronically.

Conclusions

The Purification Factory was designed in response to the high throughput purification demands resulting from the generation of large libraries of compounds using combinatory chemistry. Using four LC pumps, the Waters 2525 Binary Gradient Manager, with the MUX ZQ and four Waters WFCIII fraction collectors, the system was able to collect fractions with an average purity of 87% and an average recovery of 81%. The fraction collection parameters were set such that there was a compromise between recovery and purity. To increase one would have meant decreasing the other.

Throughput was enabled by the use of the Waters ZQ with MUX technology, which allows for four sample streams to be sprayed independently into a single mass spectrometer. Almost a thousand compounds were injected onto the XTerra columns with little to no change in peak shape. MassLynx software independently tracked each channel, while FractionLynx triggered the collection of fractions accordingly. Masslynx also allowed the independent control of four pumps and four fraction collectors. By combining these technologies, it is possible for only a few chemists to purify 120,000 samples in twelve months.[6]

References

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