Waters

Purification Workflow Management with Automated Gradient Strategies and Enhanced Fraction Analysis

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Introduction

In order to obtain good screening results using compound libraries, minimum purity standards are required. Typically this requirement is 85% or greater based on the area % of an LC chromatogram with a generic detector like UV, ELSD, MS TIC, or a combination of multiple detectors. If the screening compounds do not meet this requirement, purification and fraction analysis is required. Managing the flow of samples, subsequent fractions, and all the associated data through this process can often be difficult and time consuming.

This poster illustrates how a compound is efficiently taken through the purification process using the AutoPurify capabilities of Waters® FractionLynx™ Application Manager. This software allows for automation from the initial QC, through the purification, to fraction analysis. Using the target's data from the analytical results, the appropriate purification method, if necessary, is selected based on the user-defined purification strategy. Additionally, the selected purification method can be a narrow gradient, identified based on the analytical retention time. This provides optimal target separation of closely eluting impurities and thus improves the resulting fraction purity.

Another useful tool to streamline the process is automatic fraction analysis, which allows for fractions to be injected immediately after purification directly from the fraction bed. This improves the overall efficiency of the process by eliminating the user's intervention and improving the system's utilization, while giving high quality data. The poster shows that the results of the automatic fraction analysis are equivalent to those obtained after drying the fractions, reconstituting, and then analysis.

System

Waters® Mass–Directed Autopurification System:

Components

Waters 2525 Binary Gradient Module, 2767 Sample Manager, Column Fluidics Organizer, 2996 Photodiode Array Detector, ZQ™ Mass Spectrometer, 515 Makeup Pump, and a LC Packings 1:1000 Splitter

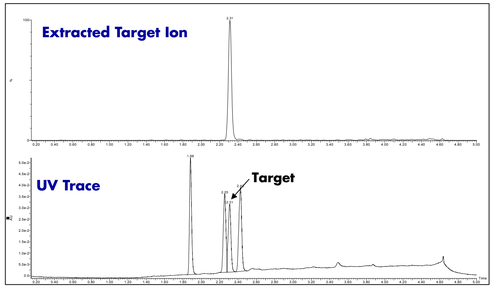
The sample was a 50 mg/mL mixture of sulfonamides in DMSO

Analytical Purity Assessment

Method

Waters® SunFire™ C18 3.5 µm, 4.6 x 50 mm
1.5 mL/min total flow water: acetonitrile: 0.1% formic acid gradient: 0-3.5 minutes 5-95% B, 3.5-3.75 minutes 95% B, 3.75-3.8 minutes 95-5% B, 5 minutes end

Analytical Results



5 µL analytical injection

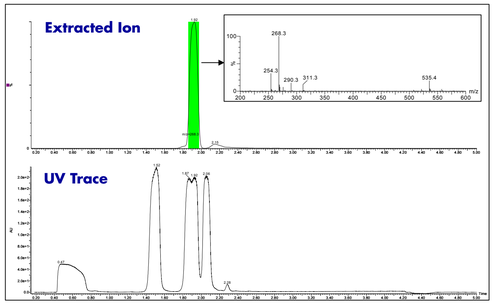
- There are 3 major impurities present with the target, one of which is partially resolved.
- Calculated purity of target is about 22% by UV

Generic Purification Results

Method

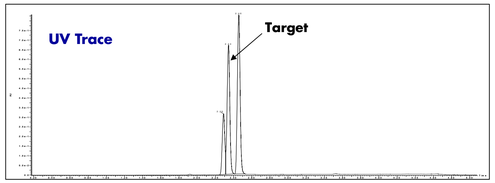
Waters® SunFire™ C18 OBD ™ 5 µm, 19 x 50 mm
30 mL/min total flow water: acetonitrile: 0.1% formic acid gradient: 0-3.5 minutes 5-95% B, 3.5-3.75 minutes 95% B, 3.75-3.8 minutes 95-5% B, 5 minutes end

Generic Purification



25 mg total load injection. An unresolved peak eluting before the target and a closely eluting peak after the target. Fraction mass spectrum shows impurities present

Fraction Analysis



Target 37% pure by subsequent fraction purity analysis, using the same method as the initial purity assessment

- *Target is not pure enough. Previously this sample would require manual intervention to achieve the required purity standards.*

AutoPurify Purification Results

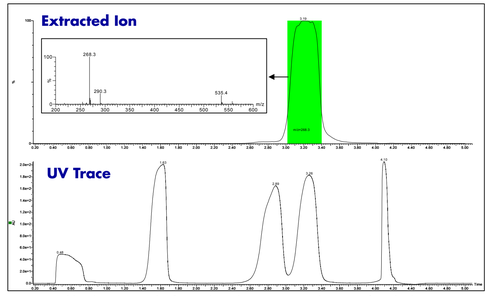
AutoPurify Purification

AutoPurify is capable of using the retention time results of the analytical data to automatically select a narrow gradient to be used for purification. The narrow methods provide for either higher purity fractionation with a shallower gradient slope or can use the same gradient slope over a shorter time.

Method

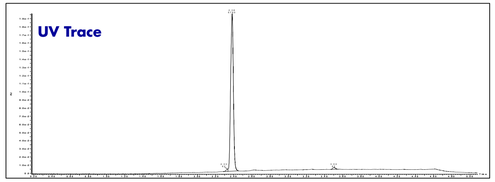
AutoPurify Narrow Preparative Gradient
Waters® SunFire™ C18 OBD ™ 5 µm, 19 x 50 mm
30 mL/min total flow water: acetonitrile: 0.1% formic acid gradient, 0-0.5 minutes 5-20% B, 0.5-3.5 minutes 20-25% B, 3.5-3.75 minutes 25-95% B, 3.75-3.8 minutes 95-5% B
5 minutes end

AutoPurify Results



25 mg total load injection. The focused gradient separates the target from both impurities. Fraction mass spectrum shows only the target.

Fraction Analysis



Target 98% pure by subsequent fraction purity analysis, using the same method as the initial purity assessment

- *Using the AutoPurify selected focused gradient, the target meets the purity requirement.*

Automatic Stages

The degree of process automation is flexible, from fully automated with no manual intervention to user controlled steps. Regardless of the degree of automation, the sample information is carried through each step to simplify the process.

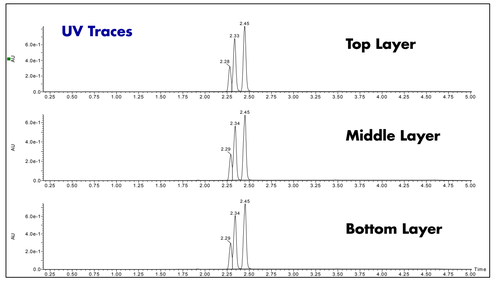
AutoPurify allows for automated fraction analysis directly from the purification bed. The benefits include:

- increased overall efficiency of the process by eliminating manual intervention and increasing the system's utilization.
- Allowing for bad fractions to be removed before dry down, saving dryer space and time.

The key to this approach is the ensure fraction analysis data is accurate and precise.

Fraction Analysis Validation

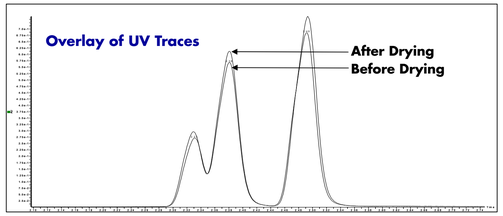
In order to validate the automated fraction analysis, the sample was aspirated at 3 different levels in the fraction tube and injected. Prior to aspiration, 2 syringe volumes (1mL) of air was bubbled through the fraction. This was performed for the generic fraction to show the impurity profile.



Generic gradient fraction analysis

Post-Collection Analysis Validation

Post-collection analysis establishes a baseline for the quality of the purification. Any subsequent changes to the target can then be attributed to other stages of the process. One example is decomposition during or after drying. This phenomenon is sample specific with multiple environmental factors to consider. In this example, formic acid was used rather than TFA because it is volatile and less reactive.



Analysis of generic fraction before and after drying by nitrogen blow down then reconstituting in DMSO.

- *No change is observed in the sample purity.*
- Other factors to consider for reducing decomposition include the mode of drying, sample storage solvents and conditions.

Conclusions

- The AutoPurify capabilities of FractionLynx allows for automation from the initial QC, through the purification, to fraction analysis.
- The AutoPurify software is also capable of automatically selecting a narrow focused preparative gradient based on the analytical results, giving greater quality purification and eliminating the need for expert manual invention to handle the "one-offs".
- Automatic fraction analysis improves the overall efficiency by eliminating manual intervention, increasing the system's throughput, and allowing bad fractions to be removed before dry down, saving dryer space and time.
- High quality data is obtained with automatic fraction analysis immediately following purification directly from the fraction vessel.
- The post-collection analysis data provides the zero-point reference for potential composition change in any of the further stages of the process.

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