Waters™

Application Note

A High Throughput Mass Directed AutoPurification System: Fraction Collection based on Mass

Chris Chumsae

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates a high throughput mass directed AutoPurification System.

Introduction

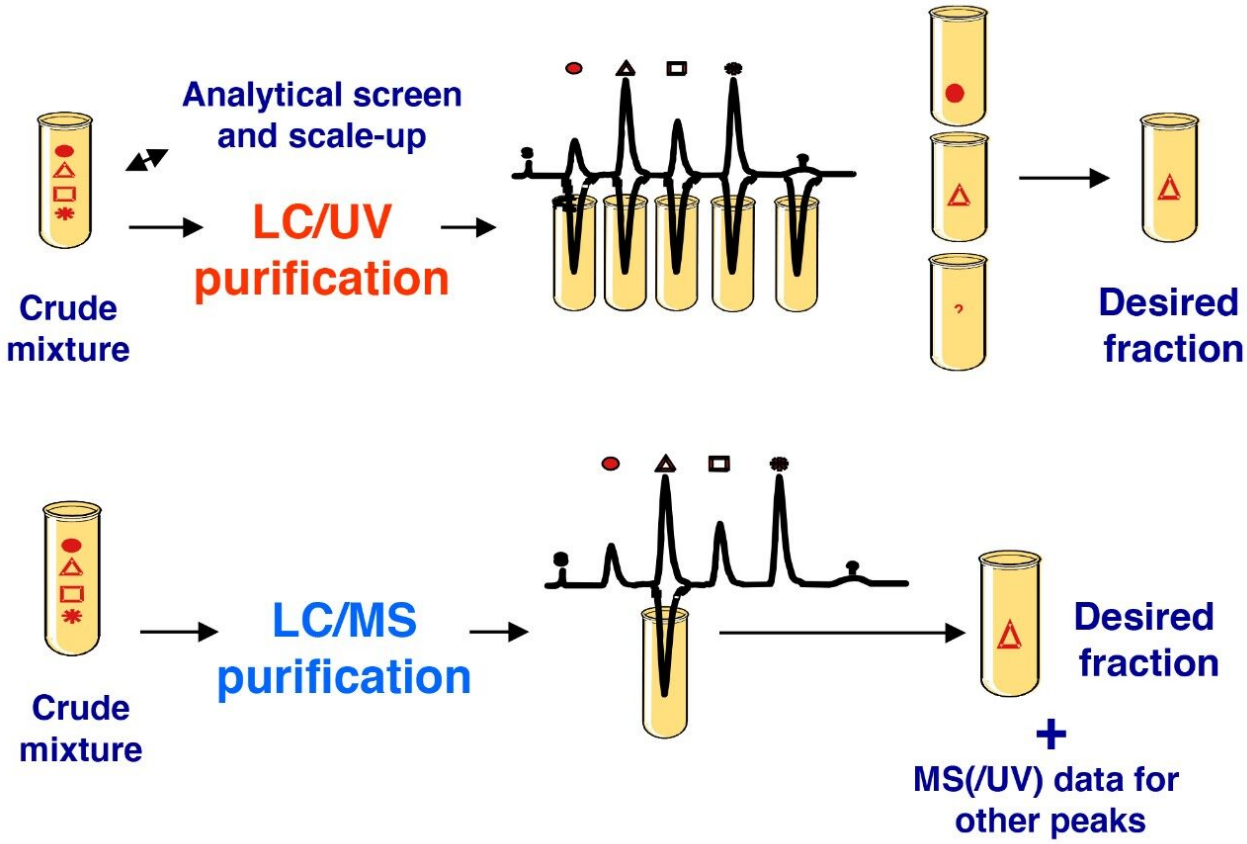
Combinatorial chemists continually face the challenge of screening thousands of samples with hopes of purifying hundreds of potential drug candidates for further investigation. The scale up of potential drug candidates yields milligram quantities which are then purified using traditional preparative or semi-preparative chromatography.

With recent developments in mass spectrometry and split flow technologies, it is now possible to purify a synthesis product using the mass spectrometer as the detector which triggers the fraction collector based on the presence of a desired molecular weight. By adding the selectivity only a mass spectrometer can provide. Chemists collect only the compound(s) of interest and eliminate analytical screening of all the fractions. This saves time, effort and expense.

Results and Discussion

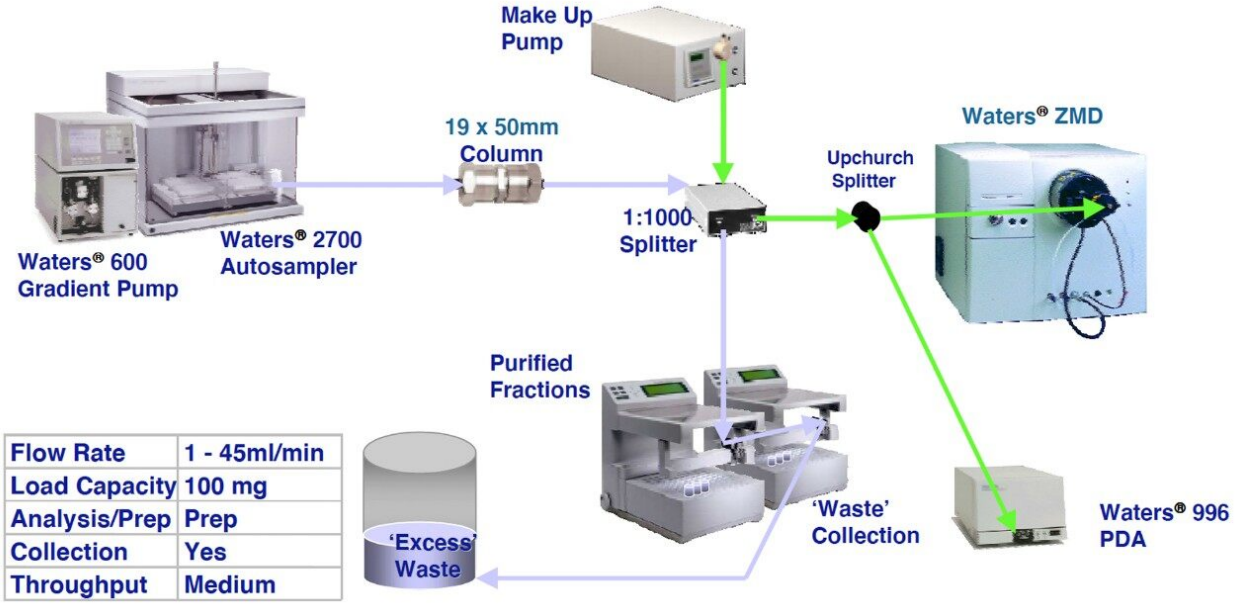
LC/UV Based Purification vs. LC-MS Based Purification

Purification based on UV absorbance allows the collection of a peak every time the acquired UV signal achieves the peak detection criteria. The chemist must then determine which fraction contains the desired compound. Mass spectrometry provides the chemist with the selectivity to target and collect specified masses only.



FractionLynx System Flow Scheme

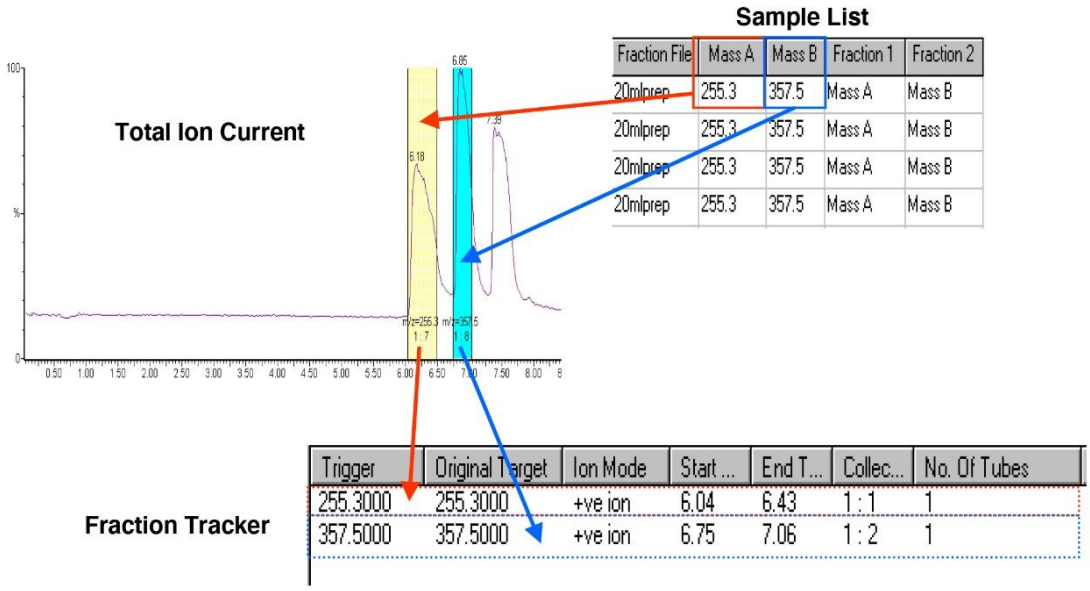
Eluting peaks flow into a novel flow splitter which splits the flow between the mass spectrometer and the fraction collector. A make-up pump dilutes and carries a very small part of the sample from the splitter to the mass spectrometer where the elution is monitored by API (atmospheric pressure ionization) mass spectrometry. Ancillary data is also collected using either UV or photodiode array detection. The majority of the sample (99.9%) is carried to the fraction collector. Advances in software allow a desired mass to be accurately collected by the fraction collector when the mass spectrometer detects the target molecular weight.



Flow Rate	1 - 45ml/min
Load Capacity	100 mg
Analysis/Prep	Prep
Collection	Yes
Throughput	Medium

FractionLynx Software

The FractionLynx user interface allows the chemist to enter up to 4 target molecular weights, as well as up to five adducts for each. Target compounds can be inputted by both numerical dalton value or chemical formula. Real time sample acquisition graphically displays regions collected from the purification. Fraction tracking is achieved using the OpenLynx browser which displays the target ion, ionization mode, collection period, fraction collector tube location and the number of tubes the compound is in.



Fraction File	Mass A	Mass B	Fraction 1	Fraction 2
20mlprep	255.3	357.5	Mass A	Mass B
20mlprep	255.3	357.5	Mass A	Mass B
20mlprep	255.3	357.5	Mass A	Mass B
20mlprep	255.3	357.5	Mass A	Mass B

Trigger	Original Target	Ion Mode	Start ...	End T...	Collec...	No. Of Tubes
255.3000	255.3000	+ve ion	6.04	6.43	1 : 1	1
357.5000	357.5000	+ve ion	6.75	7.06	1 : 2	1

Featured Products

AutoPurification System <https://www.waters.com/10007147>

WA00294, June 2000