

Development of an Automated High Performance Countercurrent Chromatography (HPCCC) Centrifuge Platform

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Background

High Performance Countercurrent Chromatography (HPCCC) is a liquid-liquid chromatography technique where both the mobile and stationary phase are immiscible liquids. Sample components are separated by differential partitioning between the two phases. For any compound the ratio of the concentration in the stationary phase to that in the mobile phase is defined as the distribution ratio, D. Achieving a distribution of approximately one provides a good balance between resolution and separation time.

HPCCC is a low theoretical plate count technique in which separation is achieved by altering selectivity rather than increasing efficiency (N). The selectivity achieved using HPCCC is orthogonal to that obtained using RP HPLC and SFC. Thus HPCCC complements these techniques and can provide an additional separation tool. The CCC coil can run in either reverse phase or normal phase mode depending on the entry point into the bobbin. In reverse phase, the outlet from the injector is connected to the centre location of the bobbin and in normal phase the sample flows from the injector to the periphery location. In reverse phase, the stationary phase is the more non-polar phase, usually the upper phase, and the mobile phase is the polar phase, usually the lower phase. As the system reaches it's operational rotation speed a dynamic equilibrium is achieved between the stationary and mobile phases thus creating a HPCCC column.

Objective

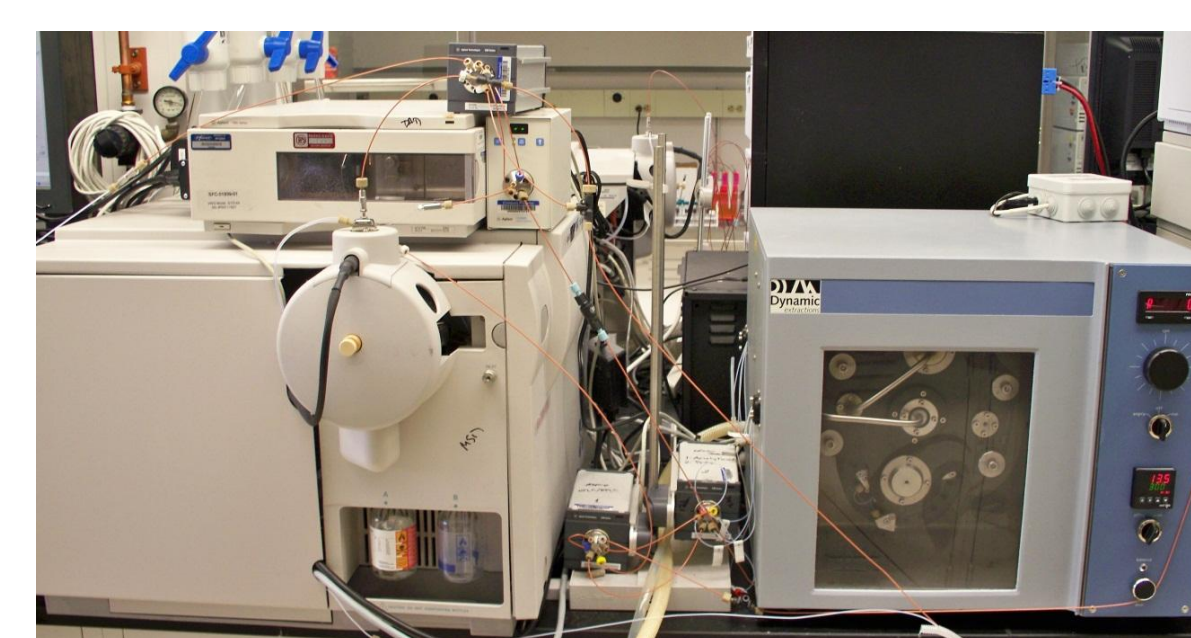
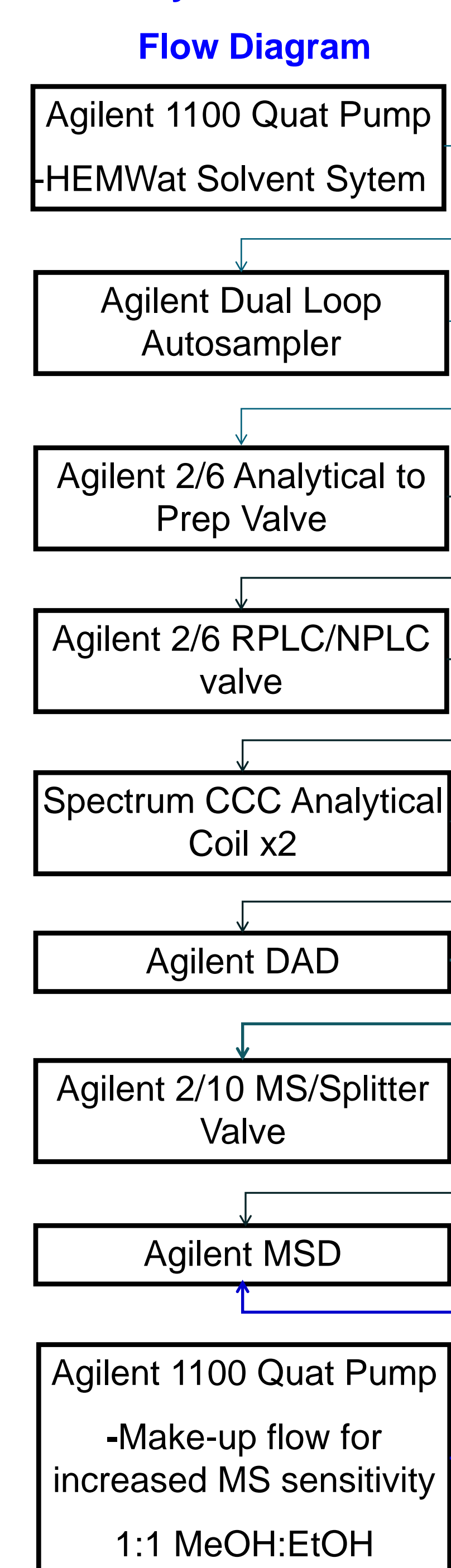
The objective of this project was to create an instrument capable of fully utilizing the potential of the spectrum HPCCC. This instrument is capable of running 4 different modes of operation. These four modes are RPLC or NPLC analytic mode and RPLC or NPLC Prep mode. Each mode is designed to take into account the solubility of the analyte and the impurity profile of the compound of interest. However, current state of the instrument in industry involves manual switching between each mode, and no mass spectrum data. It was determined during the testing phase of the instrument that automation of the instrument would be critical to the implementation into a Discovery purification workflow due to shortened timelines and low compound purity. As a part of this work, work on shorting the time required to develop a method was considered. From the onset of the project, the method development time was 6.5hrs for the primary screen of the HEMWat Solvent System looking at every other odd method. Since RPLC, NPLC, and SFC screening can reliable be done in under an hour this lends to a competitive disadvantage for CCC. A comprehensive screening panel has been built around the automated CCC.

HEMWat

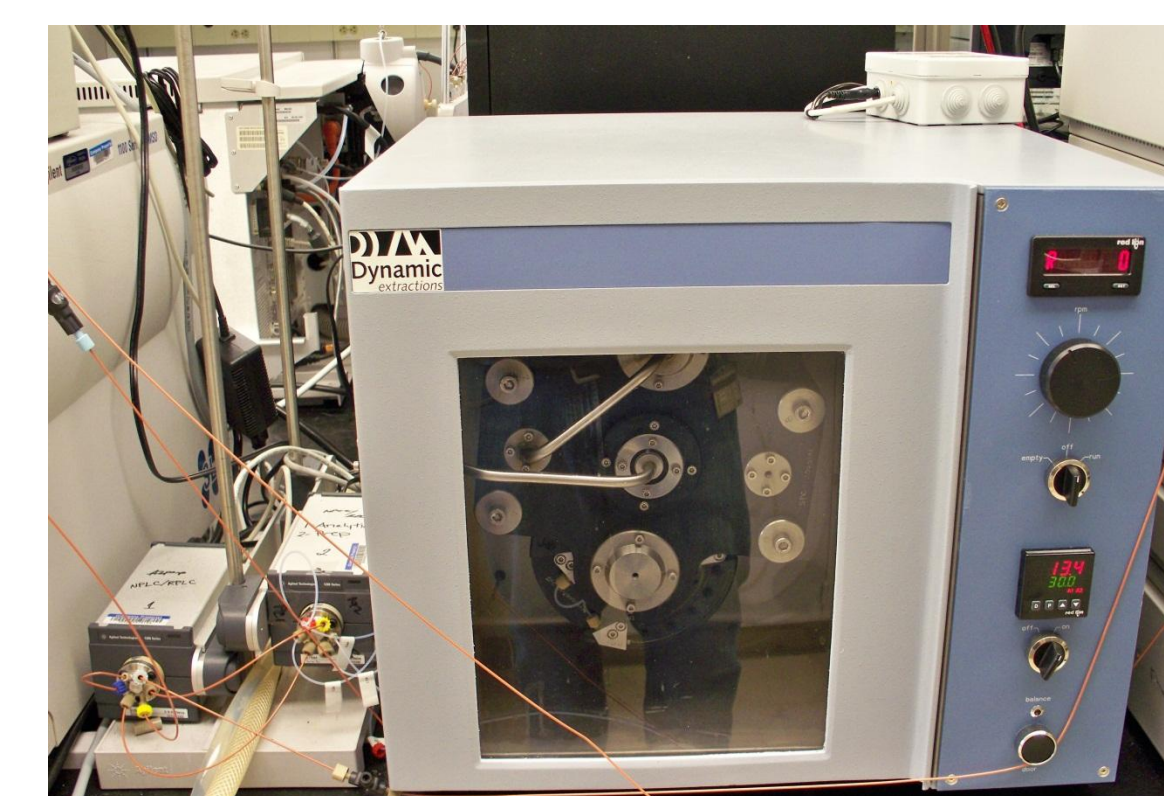
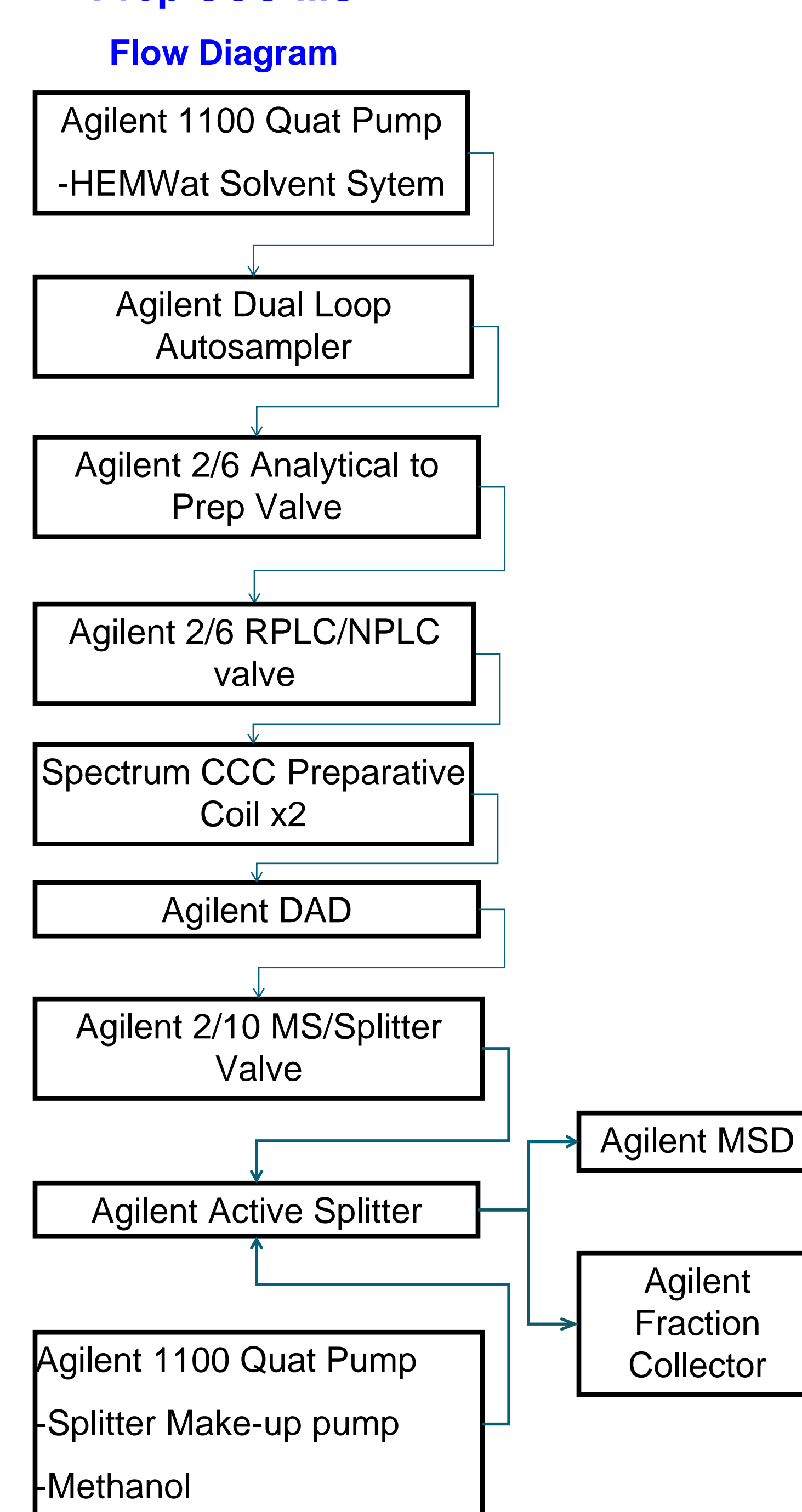
The upper phase is a mix of heptane and ethyl acetate while the lower phase is a mix of primarily methanol and water. However, the miscibility relationships between these two layers is rather complex. Heptane is immiscible with water. Heptane and methanol, however, form two phases when they are combined but are still slightly miscible with one another. The same thing is observed when ethyl acetate and water are combined. The relative miscibility or immiscibility of the solvents used in the HEMWat system determines whether or not they will form two distinct layers of solvent. There are also other parameters that are important, such as relative density of the solvents, to determine which phase will be the upper one and which phase will be the lower one.

HPCCC-MS Automation

Analytical CCC-MS Flow Diagram

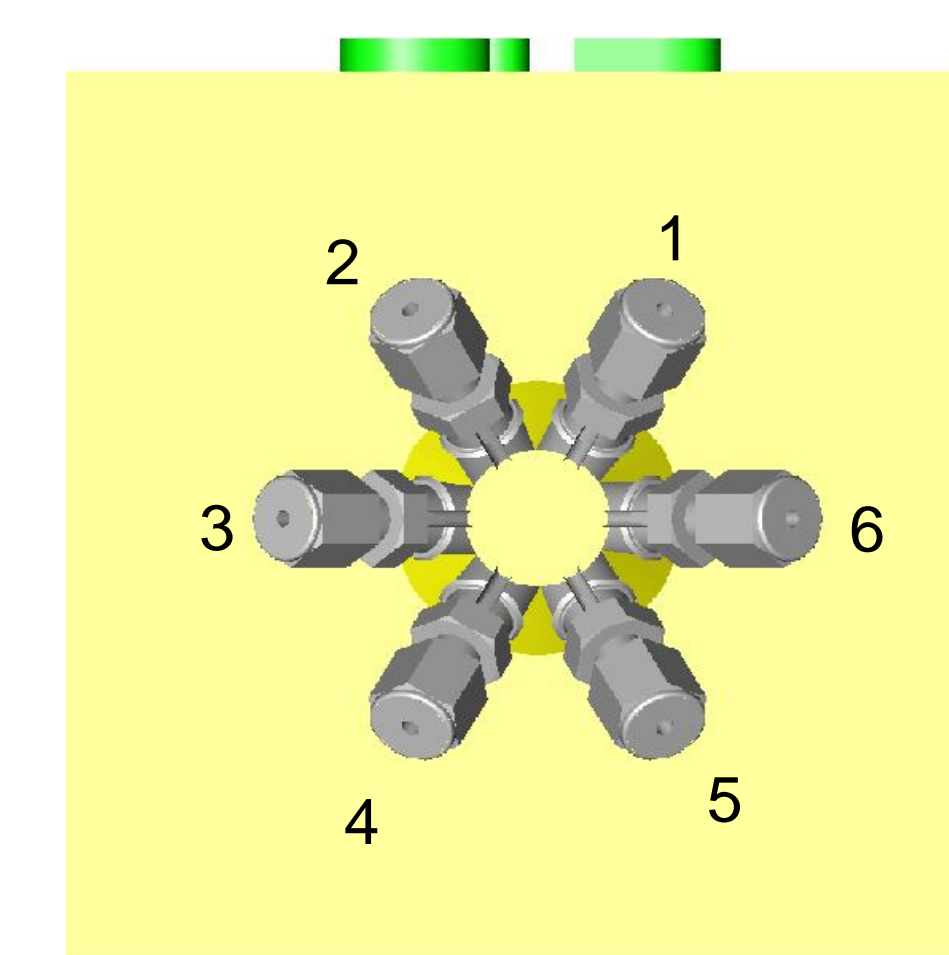


Prep CCC-MS Flow Diagram



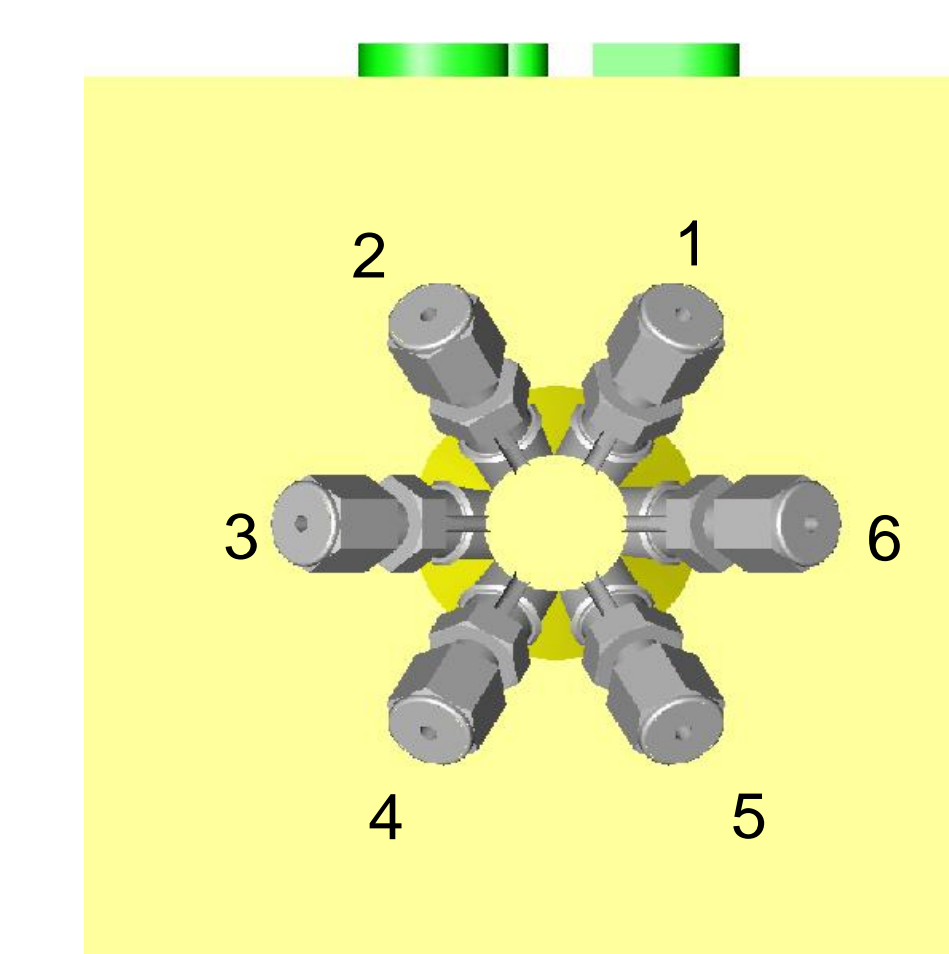
HPCCC-MS Valve Automation

NPLC/RPLC Valve



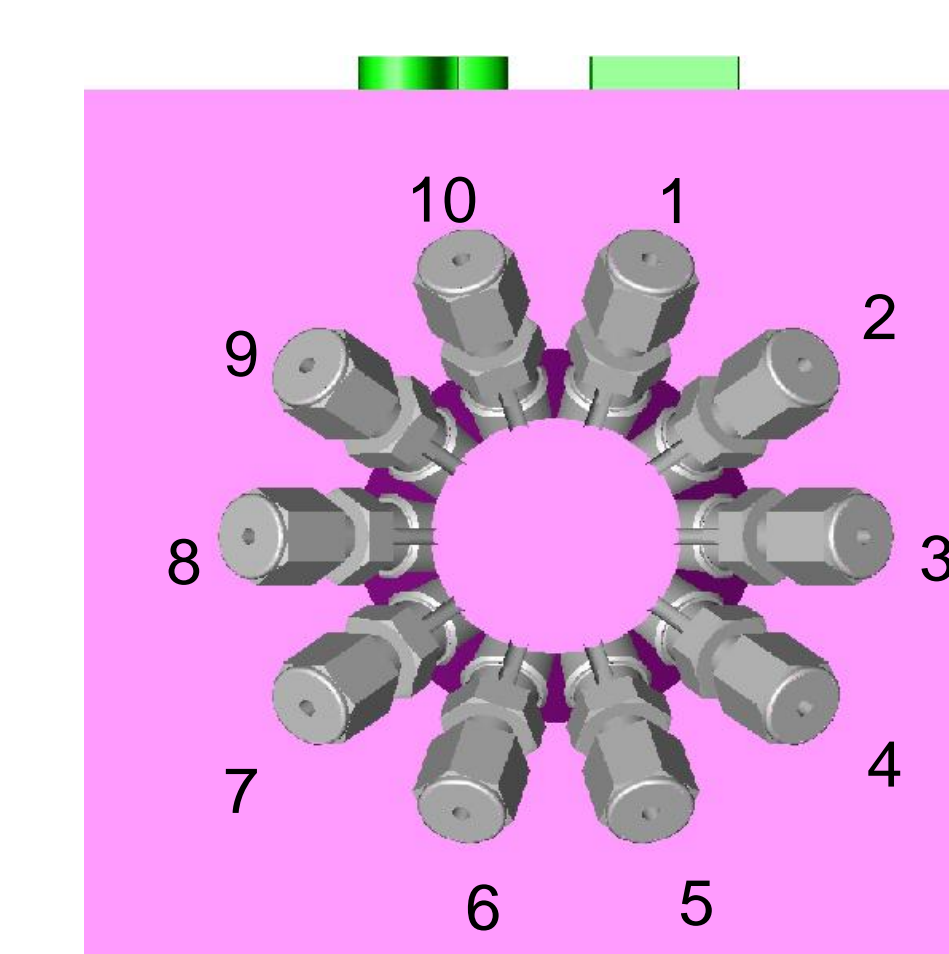
Position	Fluid Path
1	Autosampler Input
2	To Position 3 on NPLC/RPLC Valve
3	From Position 2 on NPLC/RPLC Valve
4	Position 4 on Ana/Prep Valve
5	Output to Diode Array Detector
6	Position 1 on Ana/Prep valve

Analytical/Prep Valve

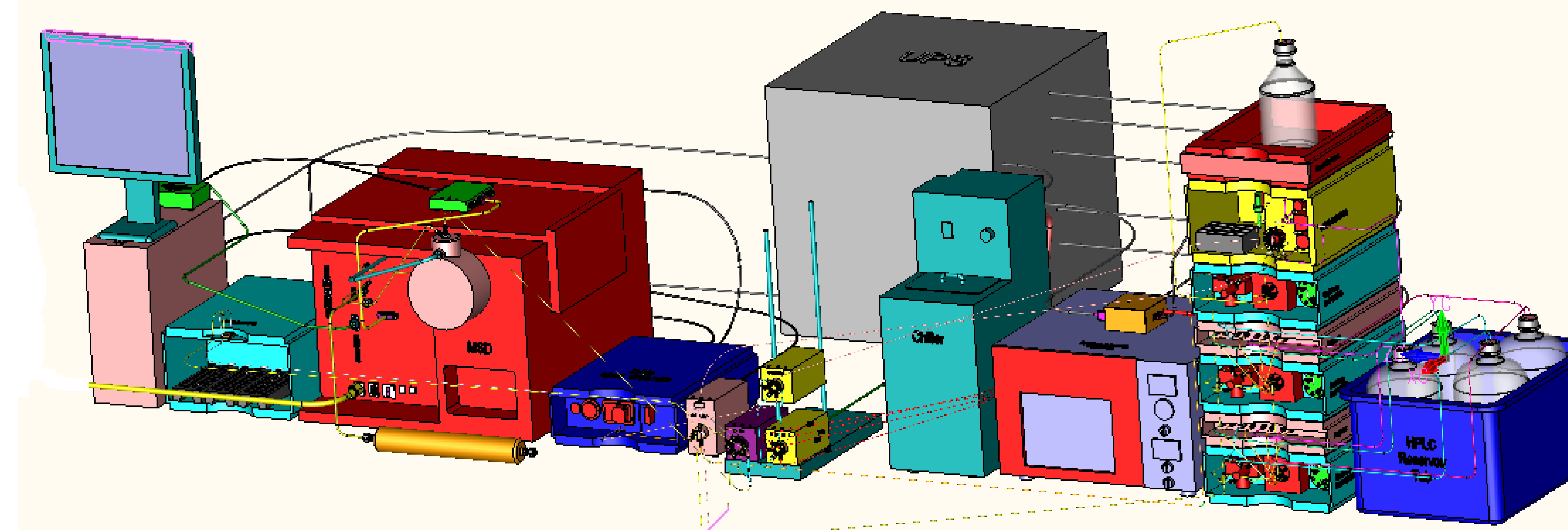


Position	Fluid Path
1	From Position 6 on NPLC/RPLC Valve
2	To Prep Coil periphery, (PP2)
3	To Prep Coil, Center CCC (PC1)
4	From Position 4 on NPLC/RPLC Valve
5	To Analytical Coil, Center (AC1)
6	To Analytical Coil, Periphery, (AP2)

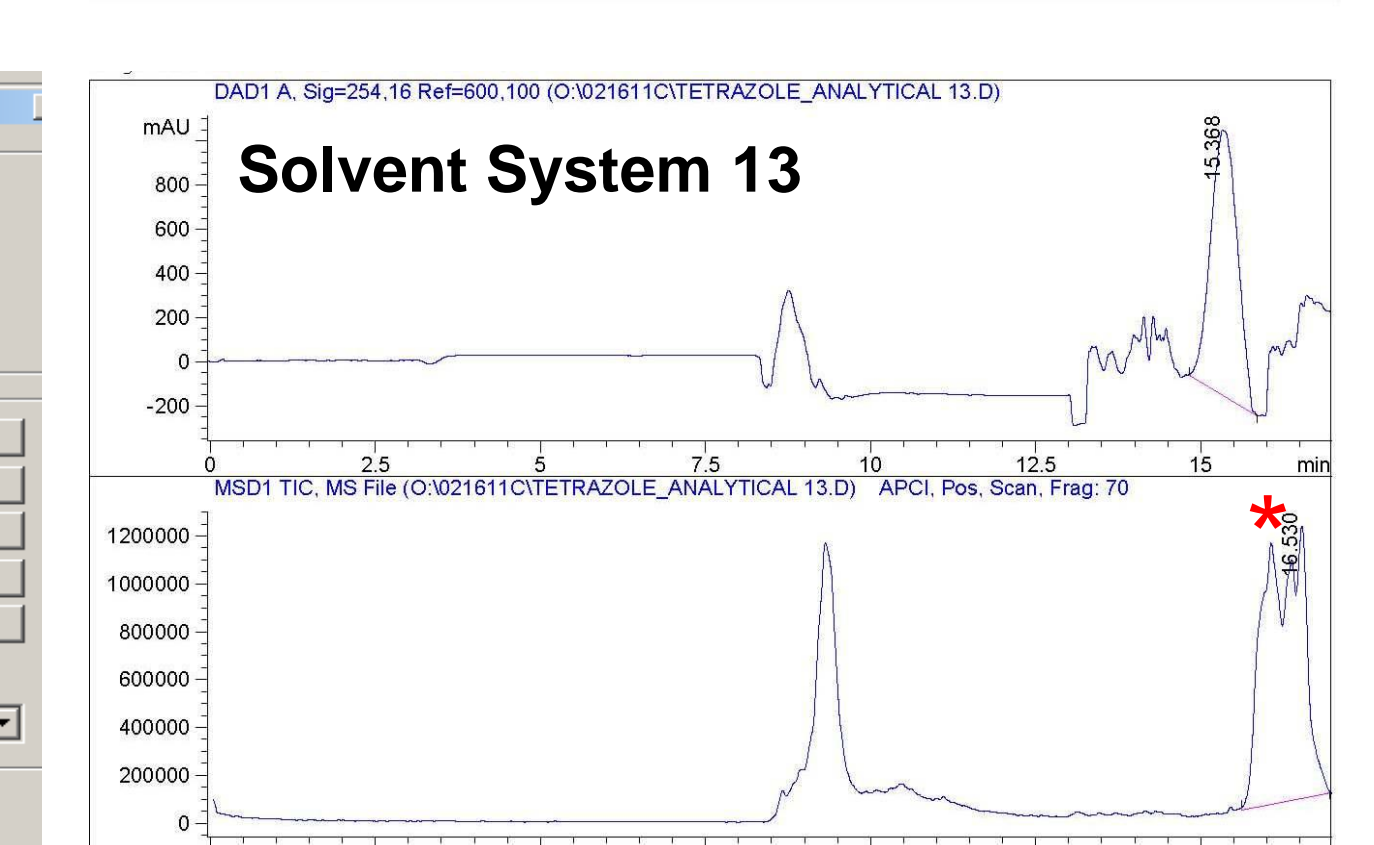
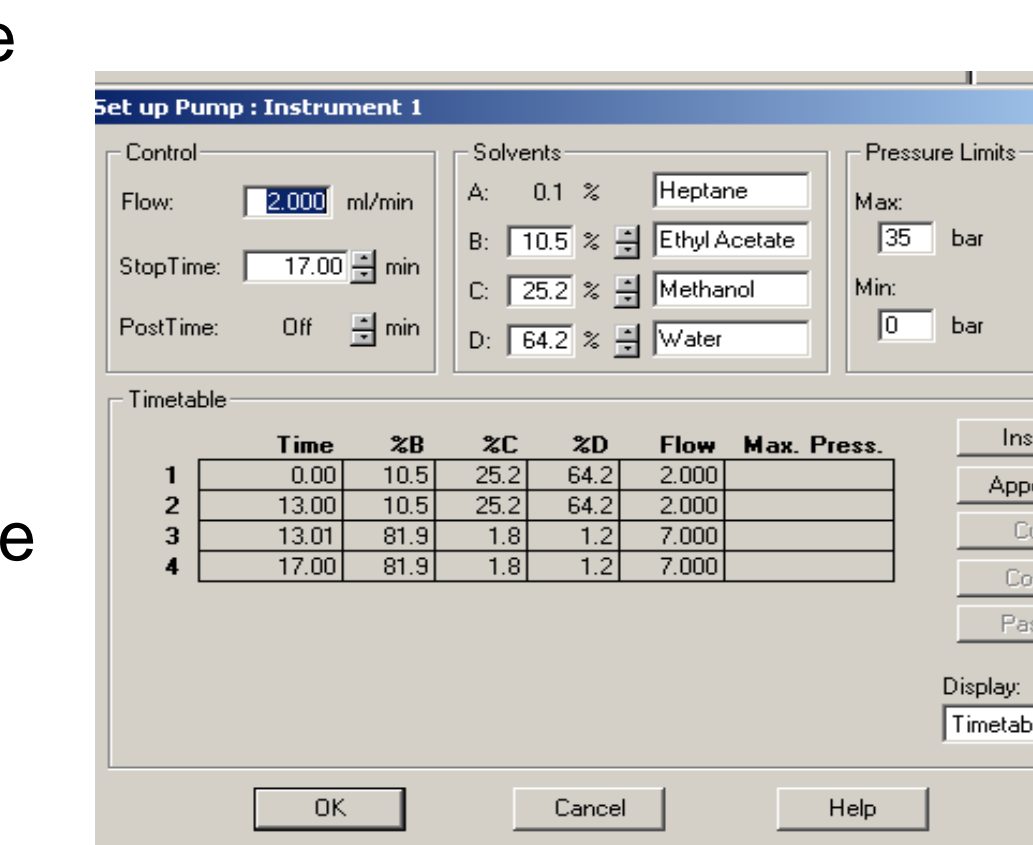
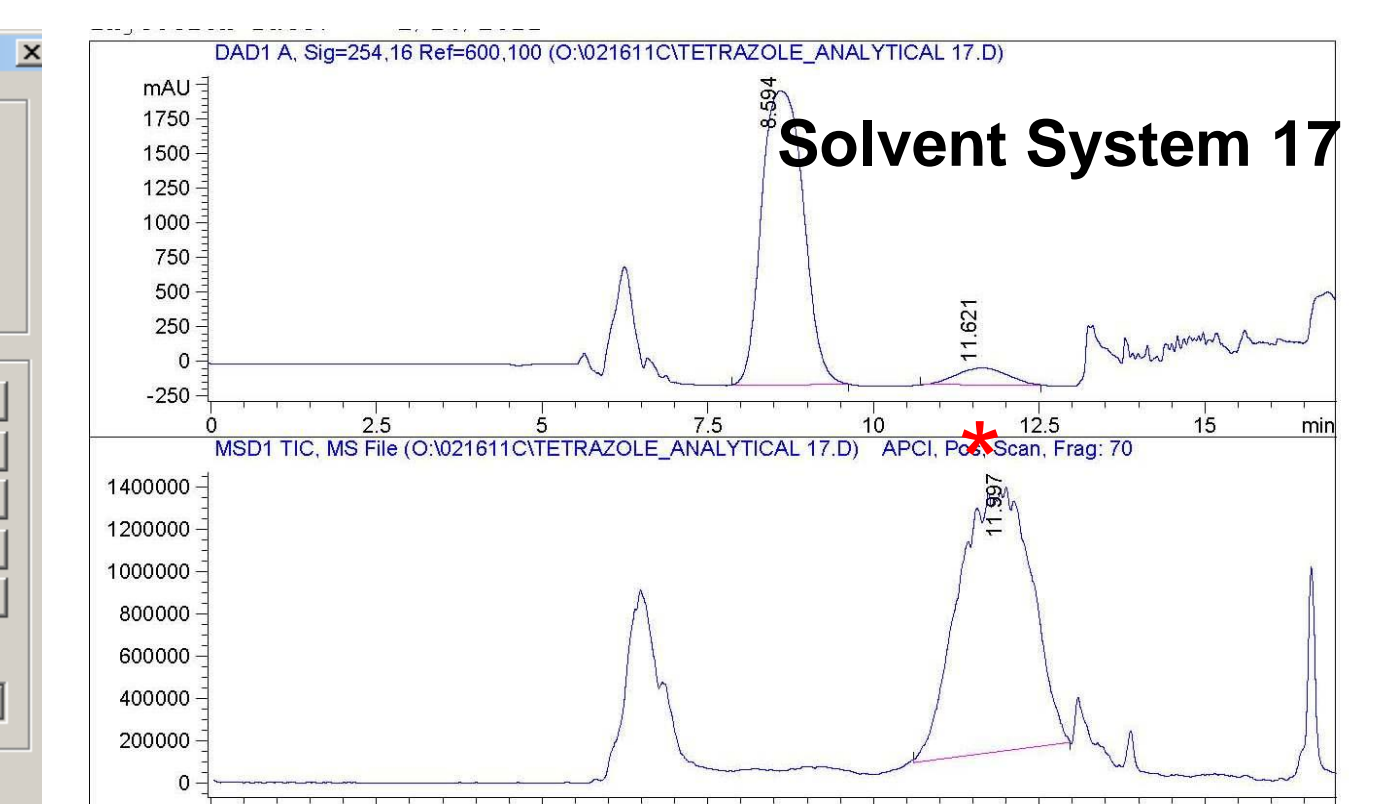
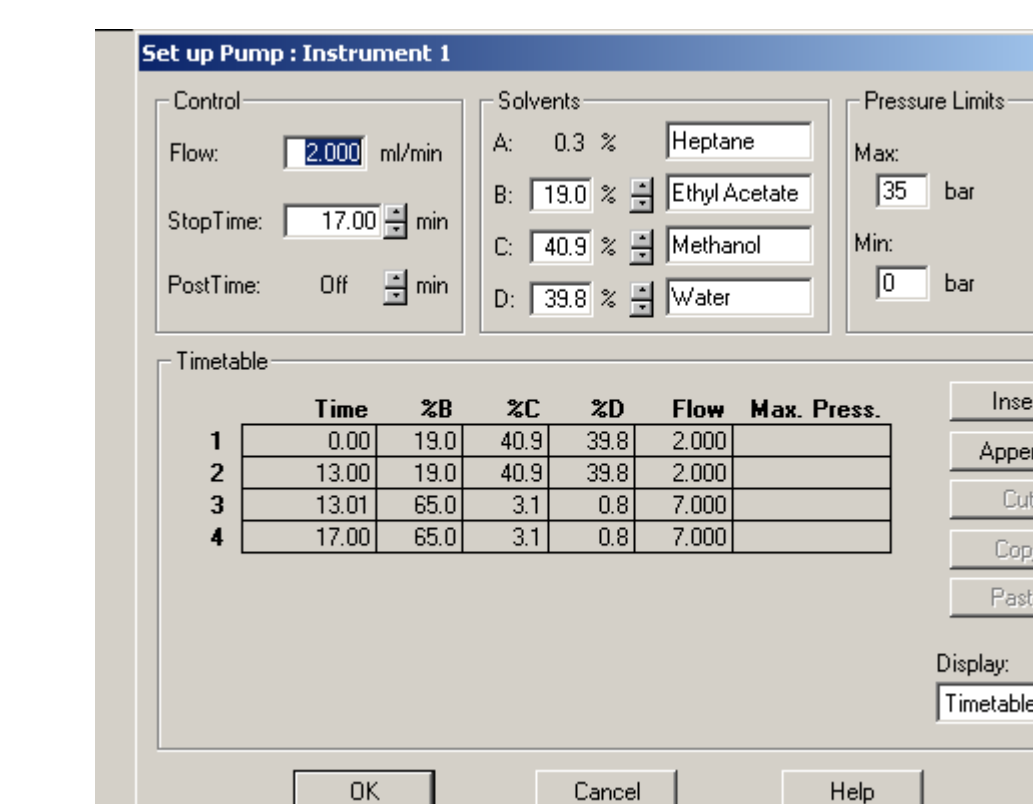
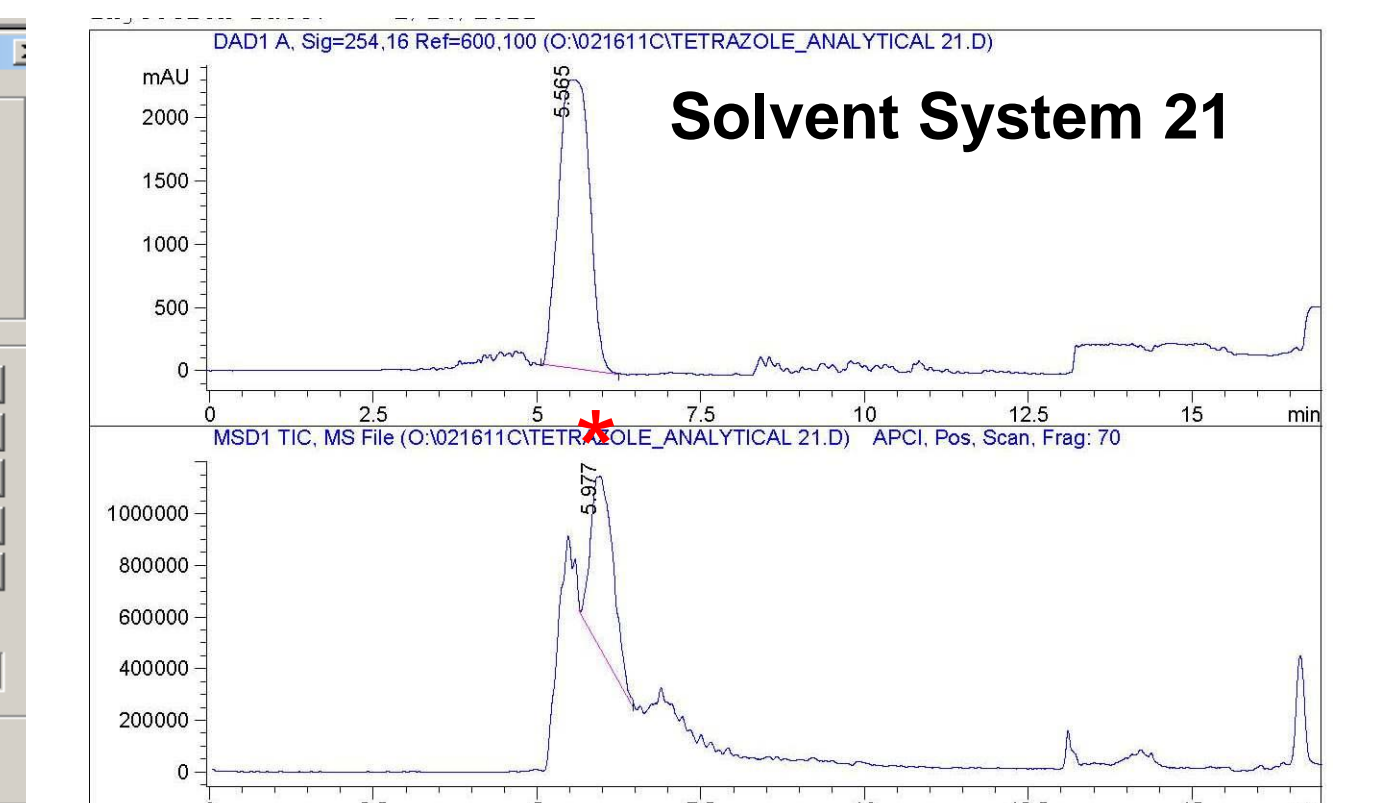
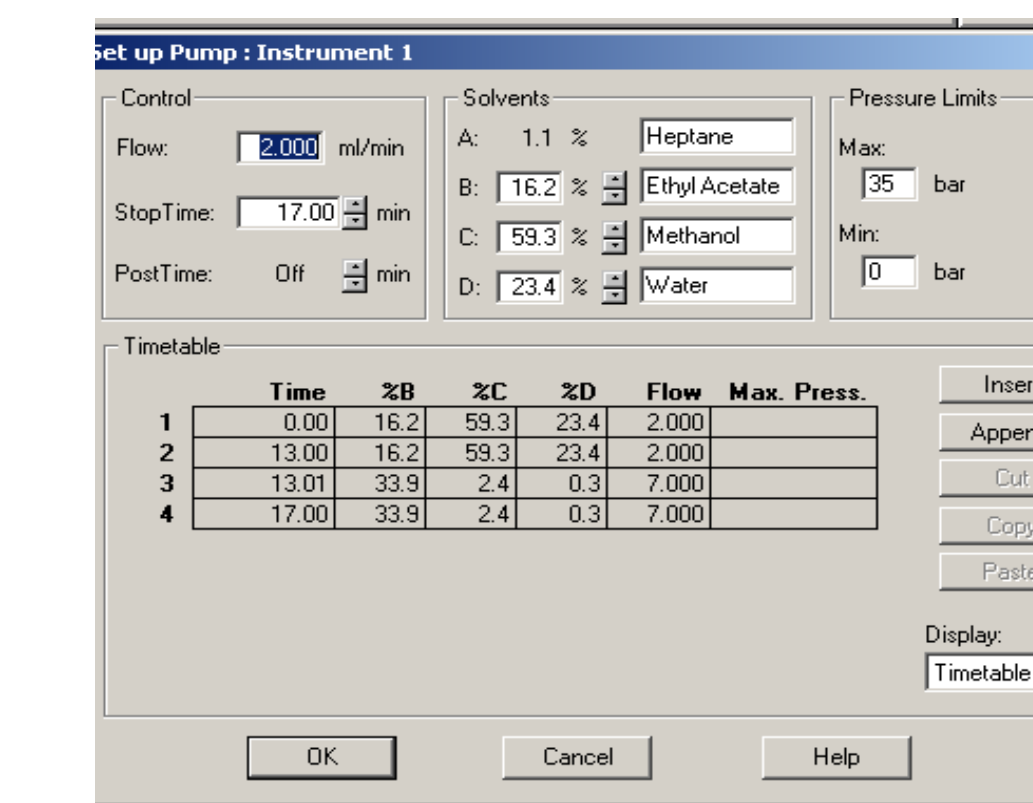
MS/Active Splitter Valve



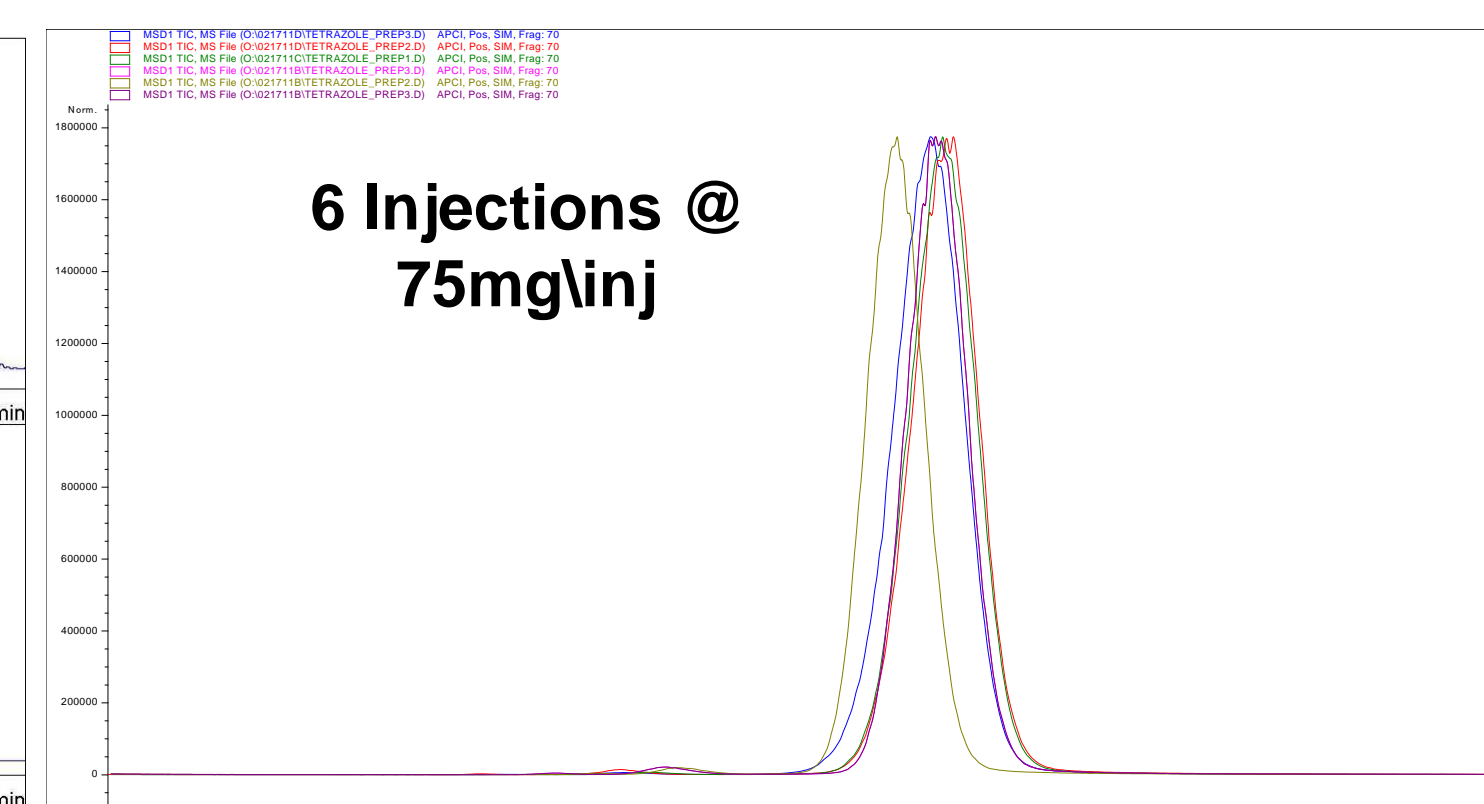
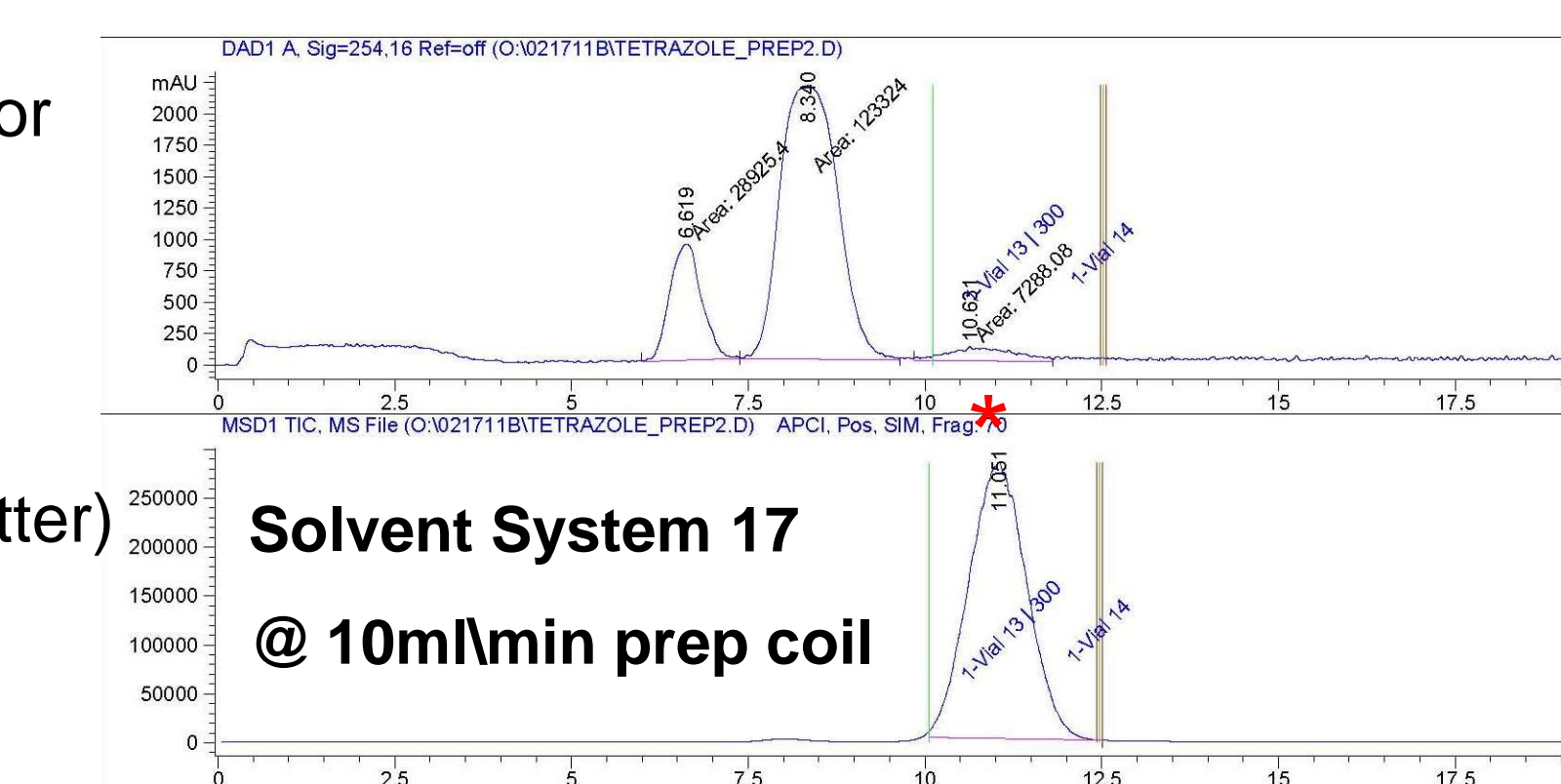
Position	Fluid Path
1	From Diode Array Detector (Through Splitter)
2	To MSD
3	Make-Up Flow(From Splitter)
4	To MSD
5	Plugged
6-9	Not Used
10	To Fraction Collector



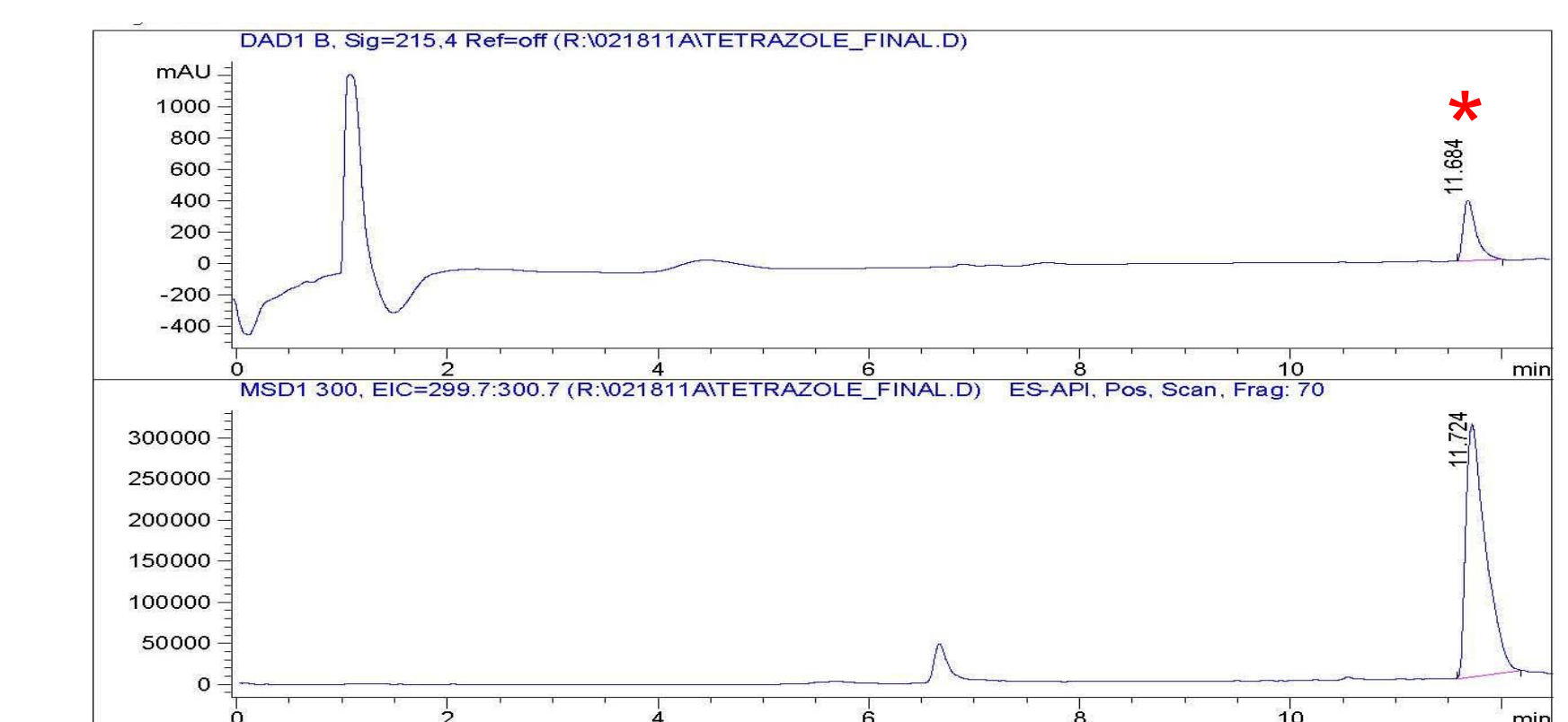
HPCCC-MS Screening



HPCCC Mass Directed Prep



RPLC-MS Post-QC



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