Optimizing Fraction Collection with LC/MSD Systems Using an Active Splitter and Delay Sensor

Technical Note

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Introduction

The extensive and growing research in genomics and proteomics has lead to many more targets for small-molecule drug candidates. There is also a corresponding growth in the number and quality of drug candidates required for screening. Reliable and meaningful biological testing depends to a large extent on obtaining drug candidates with minimal amounts of impurities and side-products. This has created a strong need for robust and efficient high-throughput systems for compound identification, purification, and quantification. With the growing trend to higher-purity compounds, synthetic chemists typically want to concentrate on organic synthesis issues and minimize the time spent on the analysis and purification of their synthesis products. The Agilent active splitter and delay sensor are an integral part of the Agilent purification/analysis platform and provide users with powerful purification flexibility while maximizing efficiency and ease of use.

The data shown in this note were generated using an Agilent 1100 Series LC/MSD SL quadrupole mass spectrometer system (G2708DA), G1361A preparative pumps, G2260A preparative autosampler, G1315A diode array detector (DAD), and G1364A fraction collector. In addition to the ChemStation software (G2710AA Rev. A.09.01) the system included Purify software (G2262AA, G2263AA, and G2264AA Rev. A.01.00) and Easy Access for Purify software (G2726AA Rev. A.01.00).

Improving Purification Productivity

The application area where the active splitter and delay sensor are most powerful is in early drug discovery in the pharmaceutical industry. With the pressure to prepare large numbers of pure compounds, synthetic-organic and medicinal chemists are searching for tools to improve productivity. The primary objectives are to:

- Collect a pure sample of synthesized products of known formula or molecular weight
- Collect microgram to milligram component amounts
- Minimize the time spent on analysis and purification of new compounds
- Reduce the cost, time, effort, knowledge, and skill required to collect the sample
- Increase chemist and lab efficiency

Figure 1 is a diagram of the system used to achieve these objectives.

Active Splitter for Flexible Operation

A very key component of this system is the Agilent active splitter. This splitter functions by taking small aliquots of the HPLC flow stream and transferring them to a mass spectrometer flow stream. This process is shown in Figure 2.

Key features of the active splitter are:

- Both the aliquot size and switch rate can be varied to adjust split ratio for each method employed. In addition, the active splitter software automatically starts and stops the valve as directed by each method.
- Different methods can have different splits without replumbing
- No inherent delay added, simplifying chromatography
- Minimal backpressure prolonging useful life of system components
- Software control of active splitter parameters and early maintenance feedback (EMF) to help schedule seal replacement

As shown in Figure 3, the software control allows easy changing of the split ratio which is then saved with the method. For example, to determine the best split ratio, the user can make several automated injections of the same sample using separate methods that differ only in the split ratio. There is no time event control of the split ratios, however it is possible to manually change

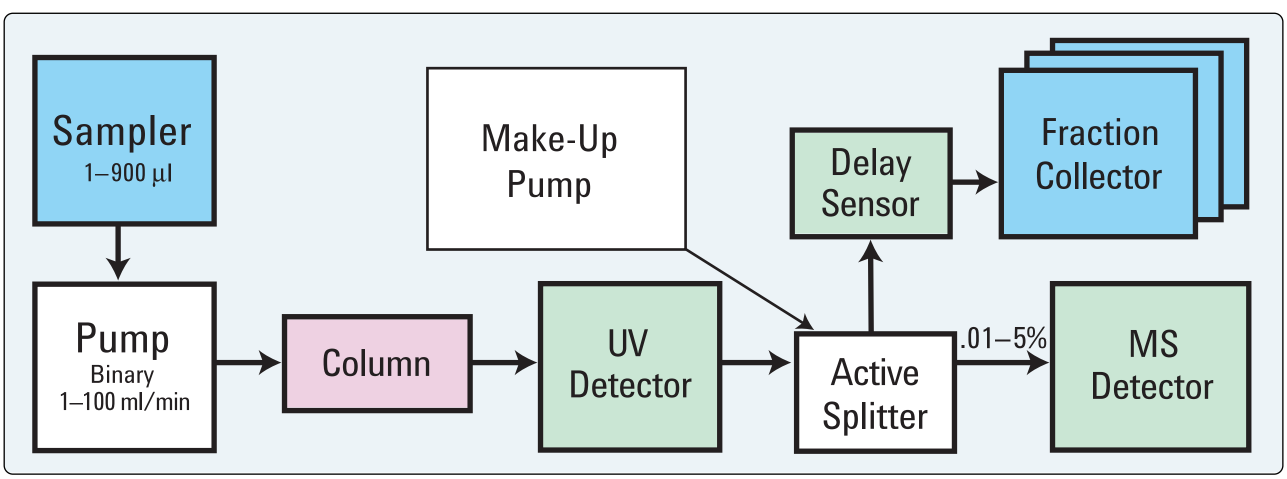

Figure 1. Purification flow diagram

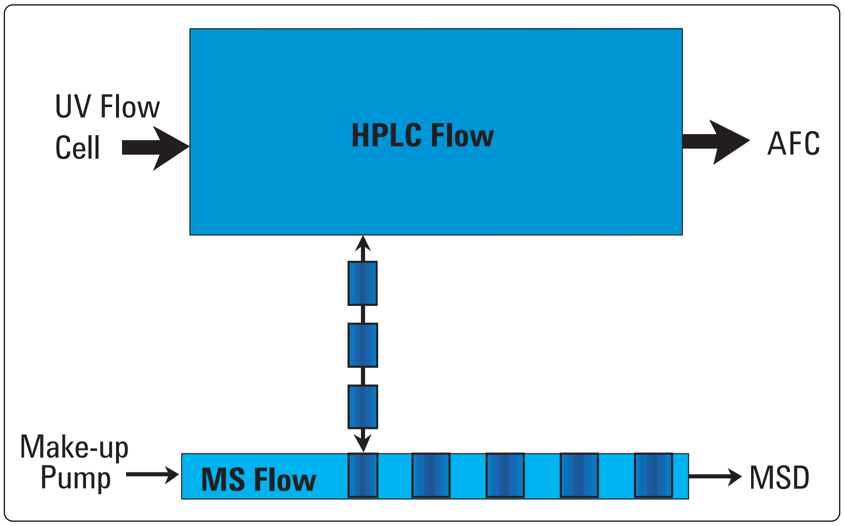

Figure 2. Operation of the active splitter

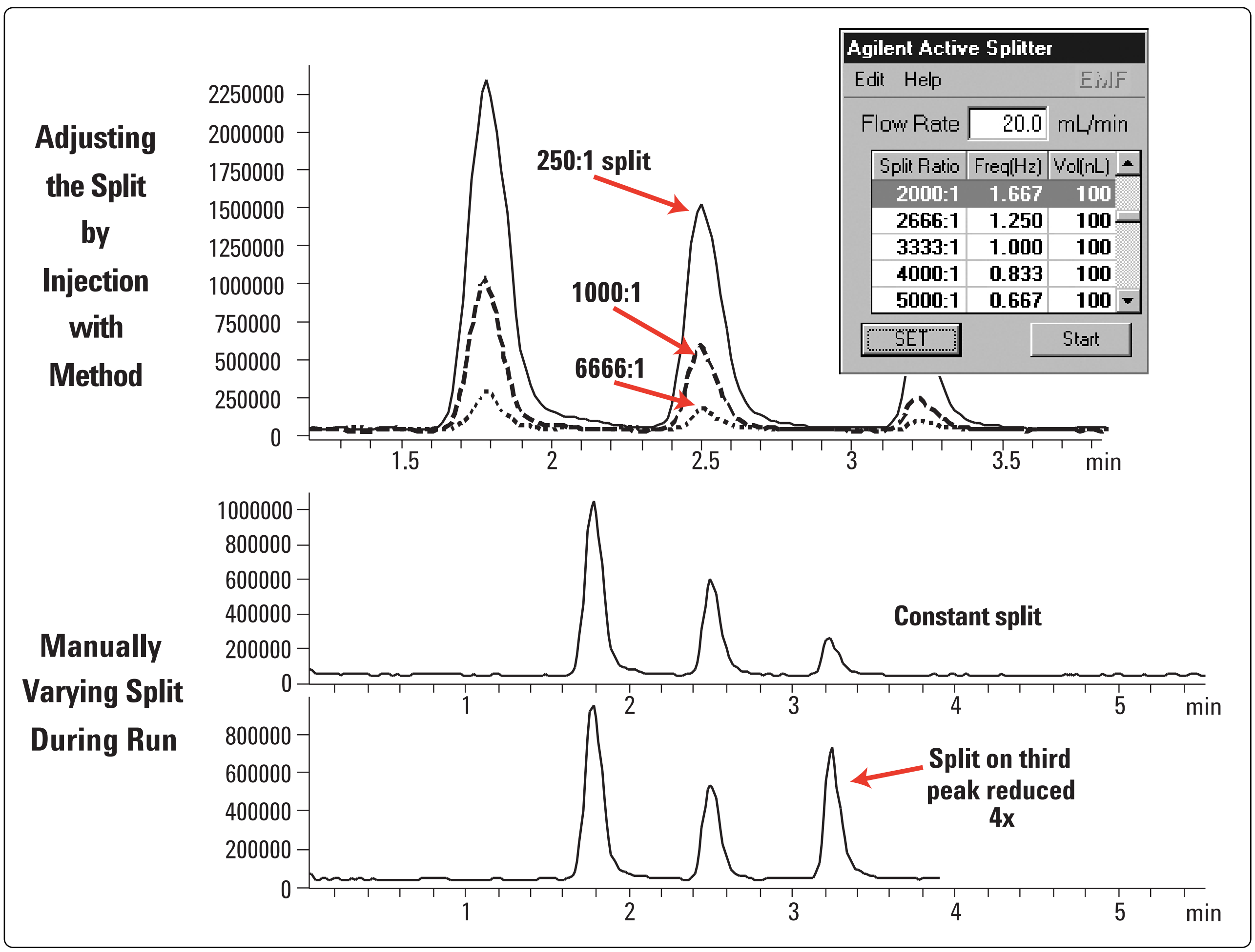

Figure 3. Adjusting split ratios

the split ratio during a run using the user interface on the active splitter itself. This can be very useful in trying to detect minor components for collection such as isolating metabolites in biological fluids, impurities in synthetic reaction mixtures and key components from natural product extracts.

EMF for Easier Splitter Maintenance

The software also features early maintenance feedback (EMF) which counts the number of cycles the valve has made and will warn the user when the seal needs replacement. This is very helpful in avoiding potential failures during an unattended run of a critically important sample. The seal replacement kit is easy to install and requires only about five minutes.

Delay Sensor Improves Systems Accuracy and Ease of Setup

Another key feature that makes the use of this system easier and more reliable is the process by which volume delays are determined. In order to avoid potential loss of valuable fractions, it is essential in mass-based systems that the peaks of interest arrive at the triggering detector(s) before they arrive at the fraction collector. This requires that tubing is in place to provide a reproducible delay from the time each detector detects a peak to the time it arrives at the fraction collector. To more easily accomplish this, the fraction collector has a built-in delay sensor (FDS). The dispensing arm positions itself over the sensor and the firmware can precisely calculate the delay volume between any detector and the fraction collector.

Key benefits of the delay sensor include:

- Accurate calculation of the time delay between peak detection and fraction collection
- Automatically saves correct delay values with the method and fraction collector configuration

For the LC/MSD, the delay sensor calculates the time between LC/MSD detection of a peak and when the peak arrives at the fraction collector. Unlike for UV detectors, this delay is impacted by changes in the split ratio and make-up pump flow as well as HPLC pump flow. Therefore, this delay needs to be recalibrated for each method flow and splitter combination. This is done using a sample containing a dye and caffeine which are injected together with no chromatographic column. The UV detector and FDS respond to the dye, while the caffeine ion at m/z 195 is monitored by the LC/MSD. The delay sensor data is monitored and saved just like any other 2D detector.

Figure 4 shows the result for this delay calibration. Shown are the three signals with the calibrant peak in each. A calibration panel pops up in the software with the proper calculated delays. The correct values then conveniently get saved with the method and fraction collector configuration.

Conclusions

The Agilent active splitter and delay sensor can greatly improve the efficiency of laboratories doing compound analysis and purification. Active splitting provides more flexibility in optimizing analysis and collection of a wide variety of compounds from different matrices. The delay sensor simplifies system setup while insuring valuable fractions are properly collected.

Authors

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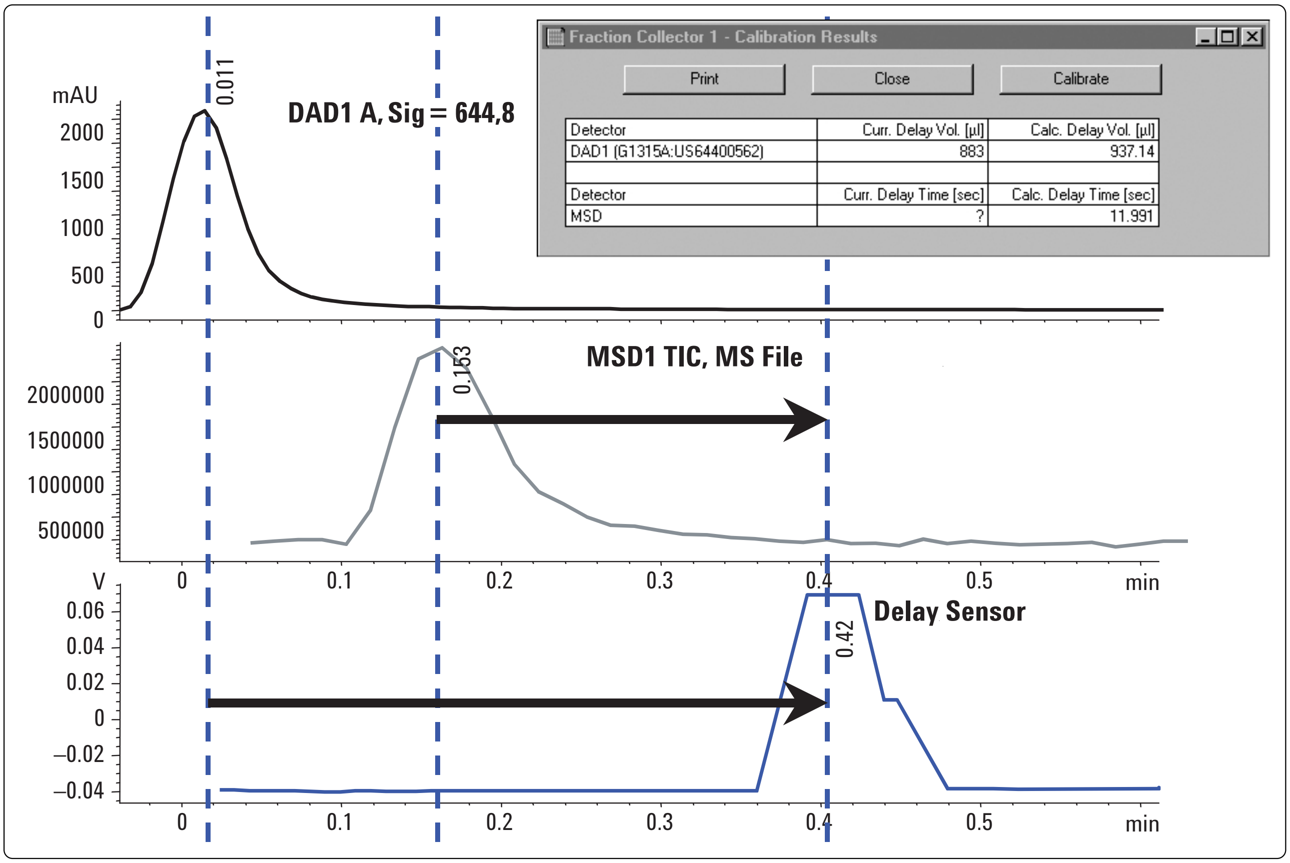

Figure 4. Delay sensor calibration

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