

# Reflections on a Third-Generation Mobile Phase Recycler for HPLC

by Al Welch

The volume of HPLC mobile phase (MP) purchased and discarded each year is about 20,000,000 L, or enough to fill two rail trains each with 100 tank cars. On average, each of the 200,000 active HPLCs pump about 2 L of MP per week. QC instruments pump 10 L each. The purchase cost for solvents is about \$20/L. Disposability, assuming it is done properly, adds at least \$30/L for a total of \$50/L. Thus, the total cost to the global society of mobile phase for HPLC is one billion dollars. If the MP is prepared manually, as is often the case, then a few hours of labor, including QC time and overhead, can be added to the \$50/L. This is a significant amount of money, especially in facilities with many HPLCs.

On a per-instrument basis, an instrument that runs for one shift per day uses about 125 L per year for a cost of \$6000/yr. An instrument in QC running 24/7 is about 500 L for an annual cost of \$24,000. Recycling of the MP reduces these costs by 50–90%. Plus, recycling reduces the environmental burden. It is in the interest of all to reduce the burden, but firms such as contract analytical laboratories and column manufacturers need to worry about being classified as contributors. Should a problem arise at a disposal site, all contributors can be held financially responsible, although typically the minor players bear much less of the financial responsibility.

## Development of MP recycling

MP recycling has been used almost since the beginning of HPLC, particularly for isocratic separations, which account for about half the assays performed. The trail was blazed by the process analyzers developed by firms such as **Applied Automation** (Bartlesville, OK), which reduced the net mobile phase consumed by returning the column effluent to the pump reservoir. Samples were seldom

larger than a few micrograms, which were diluted in at least 500 mL (~500 g) of MP for an increase in concentration of impurities of a few ppb/sample. It took many injections to have any measurable effect of the recycling of the mobile phase.

In the laboratories, the trend was to place the column out in the ambient air. Any small changes were obscured by other factors, including uncontrolled variations in column temperature. MP recycling was frequently used for instruments left running over weekends to reduce the downtime associated with start-up and shut-down. Some manufacturers advised users to never turn off the pump, except for repairs.

Total recycling works provided the concentration of the analytes in the sample is much higher in detector response than the background signal from the contaminated mobile phase. However, in total recycling, the recycled analyte will slowly accumulate in the MP. With concentration-sensitive detectors such as UV-VIS, RI (refractive index), and fluorescence, the first clue to excessive accumulation will be mysterious peaks (often negative) that arise from the vacancy effect.<sup>1</sup> In fact, if the concentration of analytes in the sample is equal to the concentration in the MP, no signal is detected. To avoid these problems, total

recycling is rarely used today. Sometimes, however, it is still employed for instruments that cannot be shut down.

## First-generation MP recyclers

As automation improved in HPLC, MP recyclers appeared and were adopted when the instrument was sufficiently useful. These split the column effluent between waste and recovered eluent that is free from the sample. The first products (ca. 1970) used timers that relied on the run-to-run reproducibility of retention time windows. While time-based control can work well in many applications, retention time can vary for many reasons, including changes in column temperature, partial failure of pump seals, column plugging, as well as stripping of the volatile components due to helium sparging. To prevent contamination of the recycled mobile phase by the sample, the window for elution of the peak needed to be about  $\pm 15\%$  wider than the peak width at baseline. This was a wide window for long runs, which reduced the potential savings.

## Second generation

Second-generation mobile phase recyclers generally employed threshold

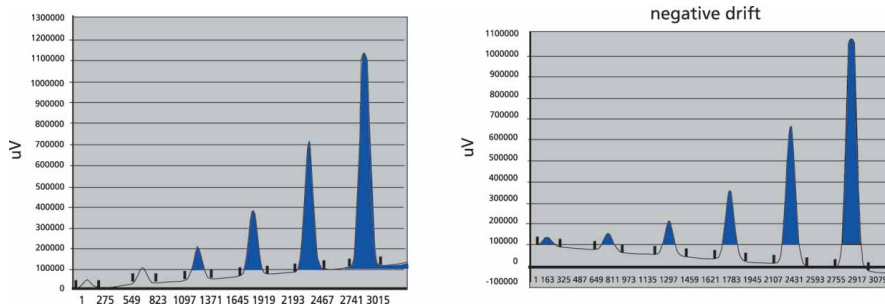


Figure 1 Threshold detection for recycling of mobile phase. The large peaks exceed the threshold level and are routed to waste. The remainder is recycled to the MP reservoir. Small peaks may not be detected since they are below the threshold. Lowering the threshold can cause problems when the baseline drifts. Drift higher than the threshold routes all MP to waste. Drift down from the threshold increases the risk of not detecting peaks, which ultimately contaminates the recycled MP.

peak sensing (Figure 1). These recyclers often successfully recovered most of the pure mobile phase. However, for some samples, the threshold could not be set low enough to see small peaks without getting lost when the baseline drifted.

If the threshold was too high, then peak detection was delayed. Upon each injection, the delay in detection sent a small fraction of the leading edge of the large peaks back to the reservoir. In addition, small peaks were not detected. If the threshold was too low, the savings was never realized since the recycling valve never reset. This was probably the worst outcome of all since the reservoir was rapidly depleted, terminating the run sequence. Many found the anticipated savings difficult to achieve in practice. Others were frustrated with the awkward controls required by the transistor-transistor logic (TTL) implementation of the controls.

### Third generation

A few years later, TTL electronics began to be replaced by microprocessors, and sophisticated, well-proven peak detection algorithms became available from the digital integrators. Threshold setting was replaced by simple input of the width of

the widest peak. A segment of a chromatogram obtained by injecting increasing aliquots of a single component sample is shown in Figure 2. The tick marks in the chromatogram show the actuation of the recycle valve superimposed on the detector output. The marks are so narrow that they are rejected by the data station as noise.

The microprocessor enables the rapid detection of peaks and baseline in real time, as shown in Figure 3. Note the presence of several small peaks between some that are large to very large. Also, the peak shapes are not ideal. To compensate for nonideal shapes, the algorithm, as incorporated in the SolventTrak mobile phase recycler (Axxiom Chromatography, Moorpark, CA), permits the user to select a return-to-baseline (RTB) delay time to make sure that the baseline is really achieved. Typically, the RTB delay is set at half the peak width.

This works as follows. At about 10.5 min, a peak is sensed and the valve is diverted to waste. The peak end is detected at about 11.25 min, but the valve is not returned to recycle until almost 12.0 min because of the RTB delay. This RTB delay for a well-shaped peak provides added assurance

that only pure mobile phase is returned to the reservoir.

A small peak is detected at about 13 min. The peak slope detector shows peak end about  $\frac{1}{4}$  min later. However, before the RTB period expires, a new peak is detected. This also happens at approx. 13.6, 14.0, and 15.75 min. The valve is set for recovery at about 17 min. The scenario is similar for the next two peaks—a peak is sensed at around 17.5 min, and the peak end is around 17.9 min, but before the RTD timed out, a new peak was sensed at 18.5 min, etc.

One can argue that little MP is recovered in a busy chromatogram such as Figure 3. This is true, but many, perhaps most, chromatograms are not so busy. This particular segment of a chromatogram was chosen to illustrate how the RTD works. In practice, most HPLCs run simple samples with high resolution, plus these are often waiting for a sample to be injected. For example, QC laboratories often dedicate one instrument to a particular analysis. The instrument waits on recycle until a sample is ready for a stat analysis. After the run is complete, baseline is sensed and it returns to recycle and waits for the next sample, which might be hours later. In this case, the savings is obvious and large (often over 90%), which corresponds to tens of thousands of dollars per year.

### Regulatory compliance

Method validation often includes requirements for fault detection. The microprocessor supporting memory and bidirectional communication of the SolventTrak facilitates recoding and reporting of the valve status for each run. With this, one can go back and examine the trail to ensure that the problem is correctly diagnosed and solved.

The SolventTrak has built-in capability to send peak detection and fault activity information to a PC program in order to produce a log file for future audit needs. The SolventTrak system will include a free logging program in the future. Figure 4 depicts an example of a log file report. The log file will contain events such as peak start and end times, periodic time stamps, communications problems, and operator intervention. Logging will be automatic and require no user intervention. These log files should serve to demonstrate that

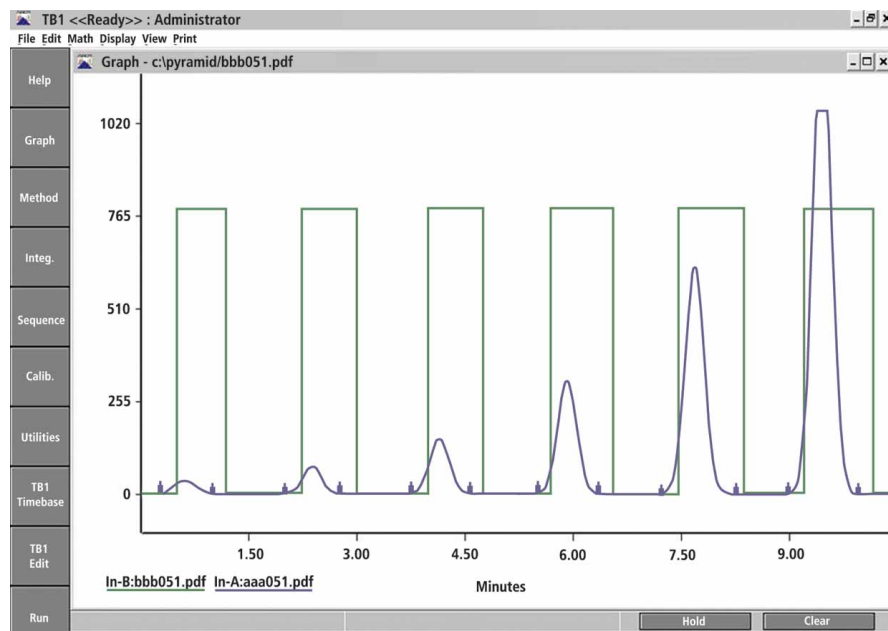


Figure 2 Chromatogram showing the placement of valve actuation for recycling of pure mobile phase. The tick marks at the beginning of the peak indicate peak detection. The ticks on the trailing edge show the peak end by returning to baseline. The square trace shows the actual operation of the recycle valve (waste is high; recycle is low). Note the valve actuation is delayed by 10 sec, which is the transit time from the detector to the recycle valve. Peak detection and valve delay are used to optimize the MP recycler to the particular separation.

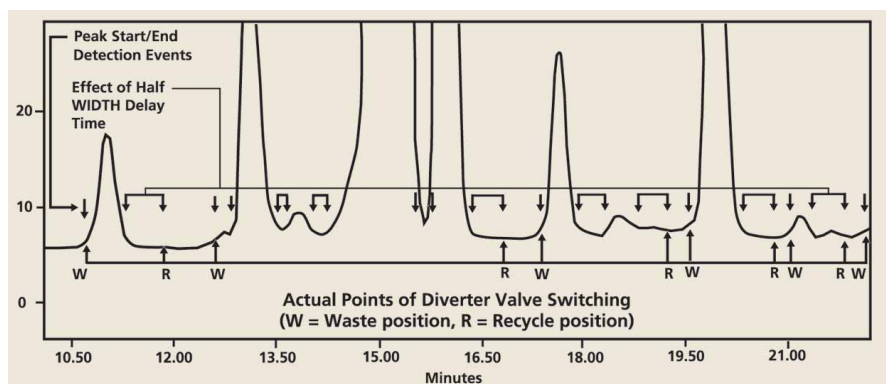


Figure 3 Illustration of peak detection used to select segments of the chromatogram suitable for recycling the mobile phase. To ensure that the material from tailing peaks is not recycled, the program uses a user-selectable RTB delay (see text).

the system has been doing its job of peak detection and recycling from day to day.

### Economic justification

MP recovery is usually justified on the basis of saving money. One study

shows a savings of 88–96% of MP and a dollar savings of 81% (~\$1000/yr).<sup>2</sup> The potential savings is greatest when an instrument has a low number of peaks per hour or day, either due to very simple chromatograms or low sample load, or both.

File Name: ST3250752\_11\_15\_05 log

Date: 11/15/2005 3:32:14 PM

Department: QC Lab

Description Station #1, HPLC, Echinachia QC, C-18 ODS 5 micron

User: Joe Labworker

Company: Moorpark Labs

Serial: # 3250752

Page #1

3:32:20 PM	Peak Start
3:32:49 PM	Peak End
3:33:19 PM	Peak Start
3:33:21 PM	Time Stamp
3:33:43 PM	Peak End
3:34:11 PM	Peak Start
3:34:26 PM	Time Stamp
3:34:37 PM	Peak End
3:35:03 PM	Peak Start
3:35:31 PM	Peak End
3:35:33 PM	Time Stamp
3:35:56 PM	Peak Start
3:36:26 PM	Peak End
3:36:39 PM	Time Stamp
3:36:48 PM	Peak Start
3:37:20 PM	Peak End
3:37:45 PM	Time Stamp
3:37:46 PM	Peak Start
3:38:09 PM	Peak End

Figure 4 Typical event table showing the time of changing the valve position. This can be useful in the initial setup of the MP recycler or troubleshooting.

Even though samples may be infrequently injected, expediency dictates that the systems be running continuously (i.e., in “ready” mode) so that the operator need only make the injection and read the response. In these situations, solvent recyclers reduce the cost of MP by more than 90% since the real run time may be only an hour per day. Thus, the recycler monitors the mobile phase and conserves it between samples. At 1 mL/min, the percent savings is at least the waiting time per day in minutes/1440. Additionally, if the chromatograms provide only a peak or two, the recycler will recover the baseline regions as well.

The benefits of MP recycling can be calculated by creating a simple spreadsheet and comparing the operation with and without MP recycling. In many cases the instrument pays for itself in a few months.

Some fear that recycling will be too much trouble. However, chromatographers are generally accustomed to using similar algorithms in setting up the automated integration to prepare a report. The first time it may take an hour or two. The second setup will require much less time.

### Environmental justification

The environmental justification involves tangible cost savings, risk reduction, and intangibles, including reputation of the firm, management, and employees. Reputation is an intangible, but it can be evaluated by the cost of replacement when the reputation is damaged. A major problem can decrease the market capitalization of a firm by 20–50%, sometimes more. Also, firms seek to compete for human talent on the basis of being a good place to work. Acting responsibly is one of the qualities often cited in ranking potential employers.<sup>3</sup>

On the tangible side, reducing the waste burden for the solvent directly reduces expense for disposal, which is more than half the estimated cost of ownership, including disposal, of about \$50/L. In smaller firms, particularly ones that use many HPLCs, such as contract laboratories, risk is classified by the level of output.

### Automated cleaning

Over the last decade, the automation of HPLC instruments has improved dramati-

ically, unattended operation is routine, and 24/7 operation is relatively common. System suitability tests can trigger column regeneration routines or purge prior to shutdown. The SolventTrak can take over and switch a second valve to provide a second mobile phase to the pump for purging and column cleanup. After the cycle is completed, the SolventTrak signals completion with a contact closure during the cleanup cycle, and all column effluent is sent to waste.

### **When not to use an MP recycler**

MP recyclers make the most sense for isocratic separations, but not all of them. Separations with small baseline periods are not good candidates. This is especially true for ion chromatography and steric exclusion chromatography (SEC). SEC has a very limited elution window, essential up to a  $K'$  of 1 or a little more for the trailing peaks. Ion chromatography with suppression essentially destroys the mobile phase chemically to improve detection. Recovery makes no sense. Plus, the chromatograms

are generally very busy, with a very low percentage of the total run time spent on baseline. However, in the case of Reagent-Free™ IC (RFIC™), as developed by **Dionex** (Sunnyvale, CA), baseline segments of the column effluent may be pure enough to recycle, even if a gradient is used.

Of course, even SEC is an attractive candidate if there are long periods between injections. In contrast, chiral separations often consist of only two peaks, plus the injection peak. Further, the MP can be expensive if a chiral selector has been added.

Column diameter has an effect. Capillary columns do not use sufficient MP to justify cost reduction, but preparative purification with large-diameter columns is probably the other extreme.

Gradient separations are generally not suitable for MP recycling, unless the wait between runs is long. Recovery of mobile phase from gradient runs involves more effort, but spinning band distillation can recover more than a

liter per hour of organic solvent, including methanol. Recovery of ethanol or acetonitrile is a bit more complicated since these form azeotropes with water.

## **References**

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