LC TROUBLESHOOTING

Solvent Conservation

How can you reduce mobile phase expenses?

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Occasionally I get questions from readers regarding how to reduce solvent consumption for liquid chromatography (LC) methods. A specific question recently concerned recycling the mobile phase. It has been more than 10 years since solvent recycling has been the main subject of an "LC Troubleshooting" installment (1,2), so this is a topic worth covering again. In addition to recycling, this month's discussion covers conservation through reduced solvent use and considers the economics of mobile phase use.

For the present discussion, I am going to focus on the consumption of the organic solvent used for LC and consider acetonitrile the selected solvent. I just checked the list price of acetonitrile from one major supplier: $39/L when purchased in cases of 4 × 4-L bottles; many of you will get discounted prices. If we assume that the "average" mobile phase is 50:50 aqueous–organic, the cost is approximately $20/L for the organic. The cost of the aqueous portion of the mobile phase is small, because most workers use high performance liquid chromatography (HPLC)-grade water prepared by a water purification system at a negligible cost after the system is purchased, and dilute buffers are relatively inexpensive. Another, often hidden, cost is disposal of the mobile phase waste stream. One laboratory I know of pays approximately $2.50/L to dispose of mixed aqueous/organic mobile phase waste. Combining all these costs: $20/L for the organic + $2.50/L disposal + a small amount for water and buffer + 10% nonproductive use of the LC system, I will use $25/L as the overall cost of the mobile phase for a typical reversed-phase LC run.

The Simplest Technique

The simplest way to conserve mobile phase is to reuse all of it. Just take the waste line from the detector and direct it back into the mobile phase reservoir. Of course this works only for isocratic methods, for which the mobile phase composition is constant. At first this might seem like you would be contaminating the mobile phase and generating spurious peaks in the chromatograms, but closer examination of the process will expose the error of this assumption. Each injection will add a small amount of the sample to the mobile phase reservoir — at most a few micrograms. If the total mobile phase volume is relatively large (I recommend 1 L) and stirred to maintain homogeneity, the percentage change in the mobile phase additive (the sample in this case) is negligible. If a chemical is present in the mobile phase at a constant concentration, it will not generate a peak in the chromatogram, even if it would if injected alone. We know this from practical experience — buffers added to the mobile phase do not cause peaks in the chromatogram, but if the buffer were injected directly, a peak might be observed. Similarly, a constant small amount of sample compound in the mobile phase will not generate a peak either.

Three precautions need to be considered if you choose to undertake this simple recycling technique. First, mobile phases will not last forever. Gradually, the more volatile components of the mobile phase will evaporate, resulting in

a change in mobile phase composition. If you limit the use of a batch of mobile phase to a week or two and keep the container almost sealed (for example, leave a 1-mm vent hole in an otherwise tightly sealed cap), evaporation should be minimal.

A second problem is that some mobile phases support microbial growth, so extended use of the mobile phase will allow a buildup of biological contaminants. This usually is not a problem when more than 25–30% organic solvent is used in the mobile phase. If you see any cloudiness develop in the mobile phase, discard it and replace it with a new batch. Don't forget to clean or replace the solvent reservoir frit to avoid contamination of the next batch of mobile phase.

The third problem relates to a buildup of contaminants in the mobile phase. Eventually, as sample contaminants accumulate, the baseline can become noisier. For most methods, this should not be a problem, because the mobile phase will be discarded for one of the previous reasons before enough sample-related materials accumulate.

Automated Recycling

You can reduce mobile phase consumption significantly if you reuse only part of the mobile phase. There are at least two devices on the market that can assist with this (Solvent Recycler 3000, Alltech Associates, Deerfield, Illinois; and Solventrak, Antech Solutions, Waterford, Ireland). These devices contain a sensor connected to the LC detector signal and a switching valve. The sensor is adjusted so that it switches the valve to direct the mobile phase to waste when a peak is eluted from the LC column and sends the mobile phase back to the reservoir when no peaks are present. For a simple method with a garbage peak at the solvent front plus a couple of analyte peaks, it is easy to see that for most of the run time, the mobile phase can be recycled. Thus, one should be able to use one of these devices to reduce mobile phase consumption by at least half. Because only clean mobile phase is returned to the reservoir, any concern about adding contaminants to the mobile phase should be alleviated. I would still recommend that you use at least a 1-L batch of mobile phase, stir it, keep the reservoir capped, and discard the mobile phase before evaporation or microbial growth become a problem.

Distillation

Another way to reduce solvent usage is to recover the organic solvent from the mobile phase and use it again. At least one company (B/R Instrument Corporation, Easton, Maryland) produces a spinning-band distillation apparatus designed specifically for recovery of LC solvents in an automated or manual process. This should work to recover solvents from isocratic or gradient applications. I do not have any experience with this device.

Change the Column Diameter

A very simple way to reduce mobile phase consumption for both isocratic and gradient runs is to reduce the diameter of the column. Most of us use 4.6-mm i.d. columns for our routine methods. As long as the same linear velocity of mobile phase is maintained, the same separation should be obtained on columns of different diameters. For example, changing from a 4.6-mm i.d. column to a 2.1-mm i.d. column changes the cross-sectional area by $(4.6/2.1)^2 \approx$ fivefold. For a method running at 1.0 mL/min on a 4.6-mm i.d. column, the flow rate should be reduced to $1.0/5 = 0.2$ mL/min to obtain the same retention times with the 2.1-mm i.d. column. A change from 4.6 mm to 1.0 mm i.d. is a factor of $(4.6/1)^2 \approx$ 20-fold. So one could reduce the mobile phase consumption by fivefold to 20-fold simply by changing the column diameter. A bonus is that peak height will increase by the same factor as the change in column cross-sectional area.

If the diameter-reduction route is taken to conserve solvent, be sure to consider column capacity and extracolumn band broadening. As the mass of packing material in the column (proportional to the diameter) is reduced, the maximum amount of sample that can be injected before the column is overloaded is reduced in proportion. This generally is of little concern for analytical LC runs, but you should be on the lookout for problems related to overload when you

reduce the column diameter. Classic overload symptoms are shorter retention times and increased peak tailing as the sample mass on column is increased. Check for this by reducing the mass on column — if retention increases and peak shape improves, overload is likely and you will need to put less sample on the column for best results.

For most applications, which do not push the limits of column performance, you generally can make the change from 4.6-mm to 2.1-mm i.d. columns without deleterious effects. However, the next step to 1.0-mm i.d. columns can be more problematic. As the peak volume is reduced (proportional to the cross-sectional area), the influence of extracolumn effects will become more important. The major factors in extracolumn effects are the injection volume, connecting tubing length and diameter, and the detector cell volume. For the typical routine LC method, 2.1-mm i.d. columns will perform quite well on conventional LC equipment; if you use 1.0-mm i.d. columns, you might need to modify the LC system or use one designed for smaller-volume columns.

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Change the Particle Size

Just as a reduction of column diameter can be used to save solvent, you can use smaller-particle columns for the same purpose. If two columns have the same column plate number, they should give the same separation, assuming the column chemistry is equivalent. The plate number is proportional to the particle diameter and the column length. Thus, a 150 mm, 5-µm particle column has approximately the same plate number as a 100 mm, 3.0–3.5 µm column or a 50 mm, 1.7–1.8 µm column. This means that you should be able to get the same separation on all three of these columns. If the flow rate and column diameter are the same in each case, the run times will be proportional to the length, as will the solvent consumption. So a 15-min run on a 150 mm, 5-µm column at a flow rate of 1.0 mL/min would use 15 mL of solvent, whereas a 50 mm, 1.8-µm column operated under the same conditions would use only 5 mL of mobile phase per run.

The gains from changes in particle size are not as dramatic as those for column diameter changes discussed previously. However, the gains from a change in particle size can be combined with the diameter changes. Thus, the 15-min run on a 150 mm × 4.6 mm, 5-µm column at 1 mL/min would use 15 mL of solvent, whereas a 100 mm × 2.1 mm, 3-µm column at 0.2 mL/min would use 15 mL × 0.2 × 10/15 ≈ 2 mL of solvent for a separation with the same resolution. The combination of sub-2 µm particles, and short, 1.0-mm i.d. columns will require specially designed equipment to avoid extracolumn band broadening problems.

What are the Real Savings?

Now that we've considered several options to reduce mobile phase consumption, let's take a look at the economics of the process. Before we do that, it is important to acknowledge that environmental concerns can be more important than the economics of the reduction of solvent consumption.

My assumption at the beginning of this column was that it costs approximately $25/L for mobile phase. If we use our 150 mm × 4.6 mm, 5-µm column operated at 1.0 mL/min and a 15-min run as a "standard" run, that run will cost about $0.375 in mobile phase costs. We could drop this to $0.05/run with the 100 mm × 2.1 mm, 3-µm column operated at 0.2 mL/min, or $0.006/run with the 50 mm × 10 mm, 1.8-µm column operated at 50 µL/min.

Direct recycling will save solvent, with more savings the longer you are willing to use the solvent. With the standard run, you should get 1000 mL/15 mL/run ≈ 65 runs from a liter of mobile phase without recycling. Increasing this by a factor of ten would seem reasonable

with recycling, so the cost per run would be in the range of $0.04.

Recycling with the help of either a switching system or a distillation apparatus also can reduce the overall solvent consumption significantly, but one must amortize the cost of several thousand dollars of apparatus, as well.

Finally, let's consider the impact of the reduced solvent use on the overall cost of analysis. In the laboratory I used to work in, we charged approximately $50/sample for routine LC–UV-type methods. The reason we could charge this much as a contract laboratory is that it cost our clients more than $50/sample to run them in-house. Pick any of the previous numbers and you can see that solvent costs are <1% of the total, even in the worst case. The column, although an expensive purchase (for example, $500/column) has a life of 500-2000 injections for most methods, so it contributes <2% to the method costs. If solid-phase extraction is used, at $2-3/cartridge, perhaps 5% of the cost can be identified.

So you can see that consumable items in the LC analysis account for a small part of the overall cost of analysis — perhaps 10%. Most of the cost is labor. Anything you can do to reduce the amount of labor usually will have a bigger impact on the bottom line than the solvent savings techniques discussed previously. In fact, some of the solvent savings techniques add labor, and therefore increase, not reduced costs.

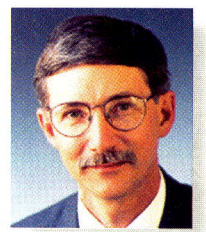
Conclusions

In my opinion, the environmental considerations are the only real justification for reducing solvent usage for LC methods - the economics just don't pencil out. However, because the change from 4.6-mm i.d. to 2.1-mm i.d. columns is so simple, and can be done for most methods without any change in LC hardware, it seems like a good choice and has financial benefits. This is the most painless way to reduce solvent consumption by a factor of five for both isocratic and gradient methods for both economic and environmental benefits.

If you have practical experience with one or more of the solvent reuse or recycling techniques discussed previously, or others I didn't mention, send me an email with your thoughts. If there is sufficient interest, I'll share these in a future "LC Troubleshooting" installment.

References

(1) J.W. Dolan, *LCGC* **10**(6), 426-428 (1992).
(2) J.W. Dolan, *LCGC* **11**(3), 204-206 (1993).



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