Maximizing Sample Recovery on the ACCQ*Prep* HP125 & HP150

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Overview

This technical note is to investigate the sample recovery and injection efficiency of different methods of injection and to discuss how modifying different method parameters can increase sample recovery using the ACCQ*Prep*, a HPPLC (High Performance Preparative Liquid Chromatography) system.

One of the most important features of a successful purification is maximum recovery of the product. This is the result of a combination of factors including:

- Ease of the separation of multiple compounds (DR_f)
- Solubility and spectral properties of the compound
- Column choice
- The instrument itself
- Method parameters

While the DR_f and solubility are unique to each separation, the best injection technique and optimizing method parameters using the Method Editor are all variables that can be used to maximize sample recovery.

There are currently three different techniques available to load a sample onto the ACCQ*Prep*:

- Manual injection via syringe
- AutoInjector needle
- AutoSampler module

In addition to facilitating automation and efficiency over manual injection, the AutoSampler and AutoInjector modules also help to standardize the loading process allowing for reproducibility and eliminating variations inherent to user driven manual injections from poor injection technique, error, or inexperience.

Additionally, optimization of the method parameters for your unique separations in the METHOD EDITOR screen can improve sample recovery. Users can choose to collect all fractions, only fractions containing peaks based upon several different detector options, or set up TIME WINDOWS to focus only on certain time periods of the separation. Further, the detector setting can be optimized, allowing it to either only monitor the separation or to trigger fraction collection if certain peak requirements are met.

As a basis for system performance, sample recovery is one of the most important attributes of a high quality HPPLC system. Many factors go into maximizing sample recovery and the following equations separated out two key factors showing how sample recovery and injection efficiency is calculated.

$$\text{Sample Recovery (\%)} = 100\left(\frac{mass\ recovered}{mass\ injected}\right)$$

$$\text{Injection Efficiency (\%)} = 100\left(\frac{mass\ injected}{mass\ starting}\right)$$

Method

Sample

A 50 mg/mL sample of butyl paraben (butyl 4-hydroxybenzoate) dissolved in methanol was used.

Method Parameters

Column: Redi*Sep*® Prep C18 20x150 mm
Sample Loop: 5 mL
Equilibration Volume: 90 mL
Flow Rate: 18.9 mL/min
Max Pressure: 4500 psi
Solvent A: Water
Solvent B: Methanol
Gradient:

Duration (min)	%B
0	70
1	70
9.8	100
2.1	100
0	70
2.1	70

Detection: UV (254 nm)
Injection Method: AutoSampler Module (PN 68- 5230-097); AutoInjector Module

Method of Analysis

A series of runs for a total volume of 5 mL over 5 injections (1 mL each injection) on either the AutoInjector or AutoSampler modules were setup.

UV detection triggers Fraction collection, while fractions in the time window are also collected. The UV detected fractions are combined, evaporated, dried and weighed for recovery. The fractions from the time window are also combined, evaporated, dried and weighed.

The residual sample in the sample vial is evaporated, dried and weighed. From this data injection efficiency and sample recovery are determined.

AutoInjector Module Probe Priming

Whether or not the tubing from the AutoInjector Module probe tip to the injection valve is filled with liquid or air may contribute to injection volume variance of the first injection of each injection series due to the differences in compressibility. Once the first injection is complete this effect is negated, as the tube is now full of fluid. To avoid this variance in the first injection using the AutoInjector sample probe, you can prime the line with your weak solvent as shown in Figure 1.

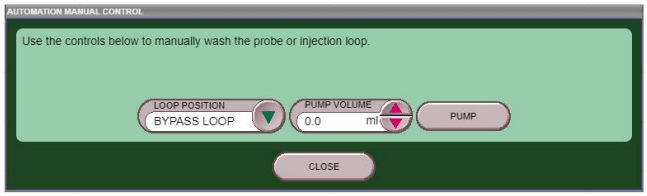

Figure 1: AUTOMATION CONTROL screen with only AutoInjector Module installed

Autosampler Wash Reservoir Priming

When using the AutoSampler Module be sure to fill the wash reservoir by washing the sample probe via the AUTOMATION MANUAL CONTROL interface as shown in Figure 2. For the same rationale as discussed above, the AutoSampler Module will automatically fill the tubing to the injection valve with wash solvent to optimize volume accuracy.

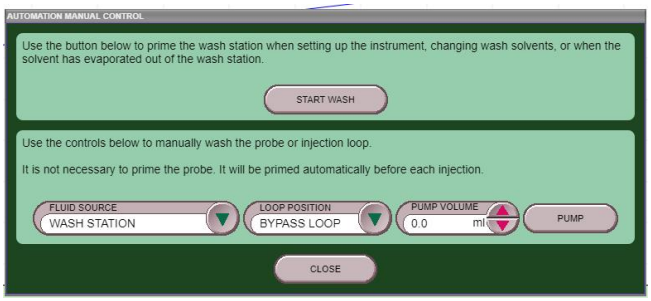

Figure 2: AUTOMATION MANUAL CONTROL screen with AutoSampler Module Installed

Results and Discussion

AutoSampler Module

Setting the AutoSampler Module to do 5 x 1 mL injections of a 5 mL sample yielded the results shown in Table 1, with Table 2 showing all the runs. Recovery from run to run varies slightly, but of most interest is the overall sample recovery and the injection efficiency. The sample recovery is corrected for the amount of sample actually injected.

Not separating out the injection efficiency from sample recovery would lead to artificially lower values for sample recovery that are unrelated to system performance. Figure 3 shows residual sample not injected after drying in the sample vial. The pattern of smaller solid forms towards the top is sample lost just due to contact with the glass. The larger solid forms nearer the bottom of the vial are from final evaporation and drying of non-injected liquid sample.

Figure 3: Residual sample in high recovery sample vial. (L) AutoSampler; (R) AutoInjector

Table 1: Example of Experimental Procedure

5 mL sample	48.255	mg/mL
1 mL injections	Mass (g)	Sample Recovery
Run 1	0.0402	90%
Run 2	0.0405	91%
Run 3	0.0411	92%
Run 4	0.0412	93%
Run 5	0.0434	97%
Remaining Sample	0.0186	
Total Sample Injected	0.2227	
Overall Sample Recovered	0.2064	93%
Injection Efficiency		92%

Table 2: Summary of Injection Efficiency and Overall Sample Recovery

Method	Injection Efficiency	Overall Sample Recovery
AutoSampler Module	89-92%	93-96%
AutoInjector Module	98-99%	92-94%

Injection efficiency of 89% to 92% were observed as shown in Table 1 using the AutoSampler Module. Due to the nature of the AutoSampler probe (larger diameter tubing with an angled cut as seen in Figure 4) some sample at the bottom may be difficult to inject, especially if the AutoSampler probe height is not set properly. This explains why there is more solid visible in the vial on the left (AutoSampler Module) versus the vial on the right (AutoInjector Module). A metal needle probe is available as an accessory for the AutoSampler Module. This "loss" from injection efficiency can easily be mitigated by adding an additional injection volume aliquot of solvent to the vial and doing an additional run.

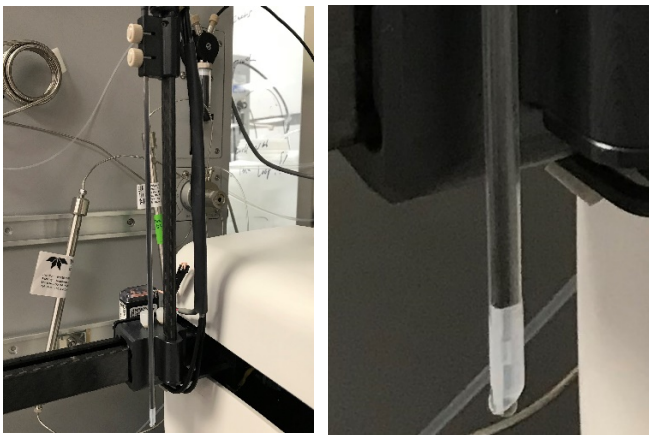

Figure 4: AutoSampler Sample Probe with closeup of tip (R)

Analysis of the sample recovery, injection to injection, shows slightly increasing sample recovery. This is best explained by concentration of the sample in the vial before injection so later runs become more concentrated as some solvent evaporates between runs. This effect will differ from solvent to solvent due to differences in volatility. This should have a negligible effect on overall sample recovery though and throughout several different series we obtained overall sample recovery from 93% to 96%. Fractions collected in the time window, but not triggered by the time window gave an additional 1% recovery, bringing total sample recovery to 94% to 97%.

AutoInjector Module

Using the AutoInjector Module with the sample needle does offer better injection efficieny of over 98%. This can be attributed to the smaller diameter metal probe (Figure 5). Recovery from the first injection vs. subsequent injections shows variance from series to series, but overall sample recovery of 92% is slightly lower to that of the AutoSampler. This can be attributed to the priming of the tube with sample fluid vs. the AutoSampler priming with wash solvent before any sample is near the valve.

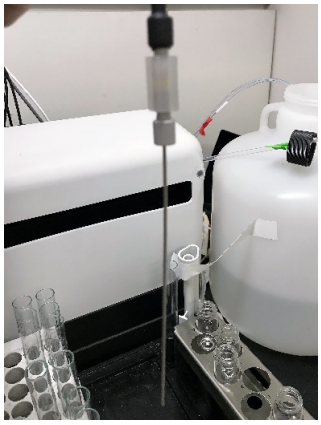

Figure 5: AutoInjector Sample Probe

Method Editor Optimization

In addition to choosing the optimal injection technique for the sample, the method parameters can be easily modified in the METHOD EDITOR screen (Figure 6). Here there are several modifiable areas to optimize recovery. In the PEAK COLLECTION section there are options to collect All fractions, only peak-triggered fractions (as further discussed below), or none.

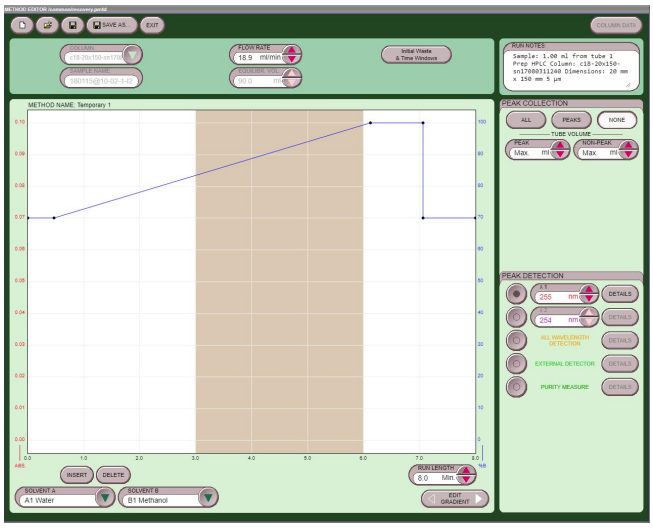

Figure 6: Method Editor screen

Additionally, there is an INITIAL WASTE AND TIME WINDOW feature. From this window, shown in Figure 7, is the option to send an initial volume directly to waste before collecting peaks or All fractions. Further, it is possible to set up to collect only certain portions of the Method separation using TIME WINDOWS if you know when the peak of interest elutes. Again, you can choose to collect the entire time window or only fractions of triggered peaks.

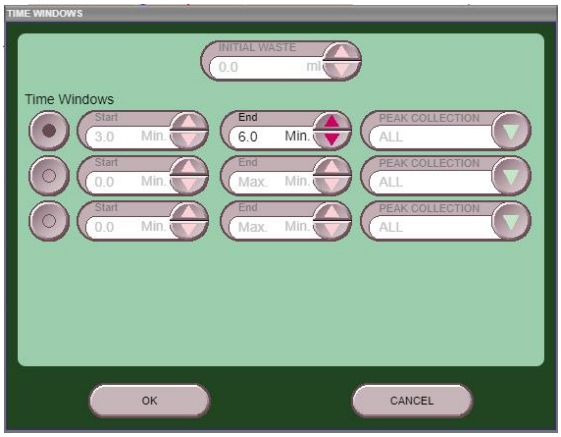

Figure 7: TIME WINDOW screen

Lastly, the method of peak detection can be modified by choosing different or multiple techniques or wavelengths. If using ELSD or an external detector you would select these in this section. Moreover, you can modify the trigger settings for each method by selecting or changing the values of the slope based on the minimum threshold. The default values are shown in Figure 8. You can also choose just to monitor the detection, thus not triggering fraction collection.

Note

If using ELSD or MS detection, sample recovery will be less as these are destructive analytical techniques, therefore the portion of the flow stream going to these detectors will be lost and unrecoverable.

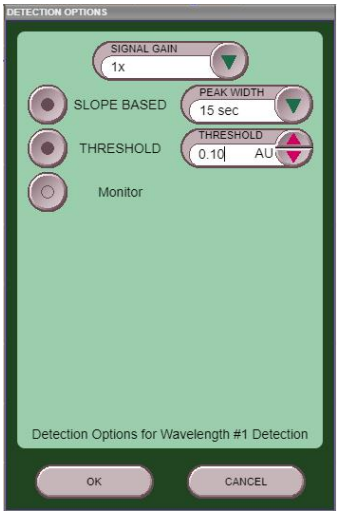

Figure 8: DETECTION OPTIONS screen

Other Considerations

Sample Loop Size

It has been determined the amount of sample lost can be influenced by the size and diameter of the sample loop and the maximum injection volume. More details can be found in Technical Note 43 *ACCQPrep Sample Loop Maximum Injection Volume*, but guidelines follow. For small loops ($^{1}/_{16}$" OD diameter throughout) sample may be lost if more than 50% of the loop is filled. For larger loops of $^{1}/_{8}$" OD tubing it has been found there is negligible loss of sample throughout the range of the sample loop volume.

For smaller loop diameters (such as $^{1}/_{16}$" OD as seen on our 100 µL and 1 mL sample loops) it is suggested not filling past 50% the loop volume in order to minimize sample loss.

For larger loop sizes (5 mL and larger, it is suggested a maximum injection of 1 mL less than the sample loop size if doing an automated injection. Therefore, 4 mL maximum for a 5 mL loop; 9 mL maximum for a 10 mL loop; and 19 mL maximum for a 20 mL loop.

Sample Vial Selection

Choice of sample vial is also a determining factor in regards to injection efficiency. The ACCQ*Prep* has sample racks for 13 mm test tubes and for high yield recovery vessels. These vessels are cone shaped to increase the height of the liquid for injection towards the end of a sample. The injection probe diameter already shows a difference in injection efficiency in the AutoInjector Module vs. the AutoSampler Module as previously discussed.

Compound Properties

Further consideration is to optimize the detector settings by confirming the maximum wavelength is set to trigger fraction collection. In the above case of butyl paraben, the triggered detection provided at 255 nm was optimal as improvement by using an expanded time window was less than 1%. Other compounds purified may have weaker UV absorbance or different λ_{max}. Doing a scouting run and finding the λ_{max} of your peak of interest by viewing the entire UV (or UV-Vis) spectrum, as seen in Figure 9, can allow you to optimize future separations. Recovery for weaker absorbing compounds might be improved by setting up time window collection or choosing other detection methods including ELSD, MS, or an external detector.

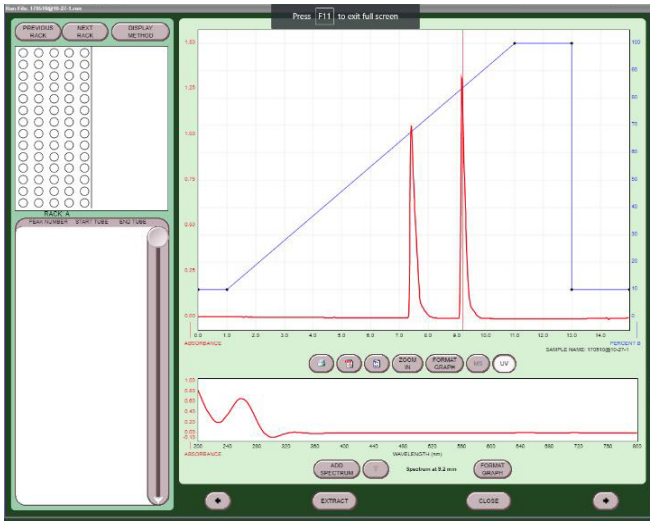

Figure 9: UV spectra at different timepoints of separation

Carryover

Carryover may occur if a compound is not very soluble in the wash solvent or proper washing of the sample probe is not performed. The AutoSampler Module has an automated wash process to minimize carryover as it washes the probe at the completion of the injection sequence with a flowing wash solvent. In this case and most cases, carryover from the AutoSampler Module is non-existent.

However, the AutoInjector Module follows a programmed sequence that must be performed by the user. It's more likely to see carryover using this method as the wash sequence doesn't involve a flowing wash source and relies on user action.

If using very concentrated samples it is also possible to contaminate the injection valve resulting in cross-over. You can flush the injection valve and sample loop through the AUTOMATION CONTROL screen (Figures 1 & 2.)

Another factor affecting carryover is the affinity of the compound for the column stationary phase. This again was not evident for butyl paraben using the C18 Redi*Sep* Prep column, as washes after runs gave clean baselines. However, depending on the compound and the loading amount, this might be encountered.

Other Areas of Sample Loss

As shown earlier, evaporation of the injection solvent could be a cause of decreased injection efficiency. Another area in regards to injection efficiency could be differing solvents (or even temperature) of higher or lower viscosity affecting injection volume accuracy.

Additionally, the reproducibility of the AutoInjector and AutoSampler injection methods provide superior results versus manual injection, as the process is more consistent from injection to injection as discussed in Technical Note 40 *Guide to Different Loading Methods for the ACCQPrep*.

Conclusion

The ACCQ*Prep* offers several ways to improve sample recovery and injection efficiency. Default detection triggered fraction collection offers better than 90% sample recovery without any optimization. Injection efficiency is around 90% for the AutoSampler Module and near 99% for the AutoInjector Sample probe. Methods to mitigate sample loss and improve experience are described.

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