ACCQ*Prep* HP125 Minimum Injection Volume

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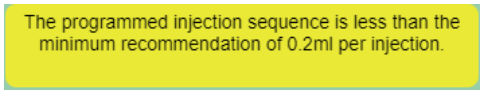
Chromatography Technical Note TN44

Overview

The ACCQ*Prep* HP125 is a HPPLC (High Performance Preparative Liquid Chromatography) system which has a minimum injection volume limit. Currently, injections of volumes below 0.2 mL are possible (down to 10 µL), but result in the following warning:



Sample injection volumes below 0.2 mL may be desired in order to preserve sample during method development. This Technical Note will examine the lower limits of sample injection volumes.

Method

Sample

Two different concentrations (1 mg/mL and 10 mg/mL each) of mixtures of methyl and propyl paraben (methyl and propyl 4-hydroxybenzoate) dissolved in 1:1 water:methanol were used.

Method Parameters

Column: Redi*Sep*® Prep C18 4.6x150 mm
Sample Loop: 100 µL
Equilibration Volume: 8.1 mL
Flow Rate: 2.0 mL/min
Max Pressure: 4500 psi
Solvent A: Water
Solvent B: Methanol
Gradient:

Duration (min)	%B
0	30
1	30
9.8	100
2.1	100
0	30
2.1	30

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

Instrument Preparation

Due to rounding settings in the software, the only way to inject volumes less than 0.1 mL is to set up multiple injections using an AutoSampler Module or AutoInjector Module's sample probe. The minimum total volume that can be entered is 0.1 mL and the minimum calculated volume injected is limited to 10 µL. For example, in order to do 10 µL injections, you must set the total volume of the sample to inject to 0.1 mL and do 10 injections.

When using the AutoSampler Module fill the wash reservoir by washing the sample probe via the AUTOMATION MANUAL CONTROL interface as shown in Figure 1.

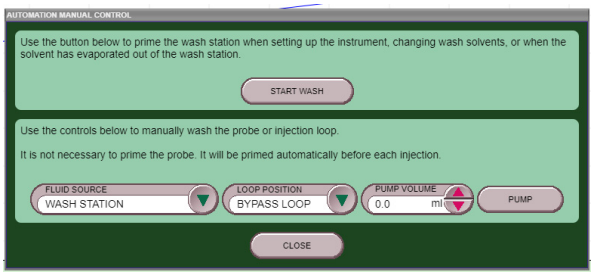


Figure 1: AUTOMATION MANUAL CONTROL screen

Data Analysis Method

PeakTrak doesn't offer a method for integration of peak area. In order to evaluate the efficiency of minimum injection volumes the pertinent chromatographic data was extracted from the run (.txt) file as described in Technical Note 45 *Exporting and Quantitating Chromatography Data from the ACCQPrep HP125*.

Using this procedure, we are able to compare the peak integration data to determine the relative standard deviation (RSD) of multiple injections, linearity of varying injection volumes and the deviation from expected integration values.

Results and Discussion

The retention times of the two compounds remains similar across the different sample injection volumes tested. Resolution between the two compounds is maintained. The RSD of retention time for repetitive injections of the same volume was less than 1.5% for all cases as seen in Figure 2, with an expected larger deviation on later eluting peaks as the sample loading volume increases band broadening.

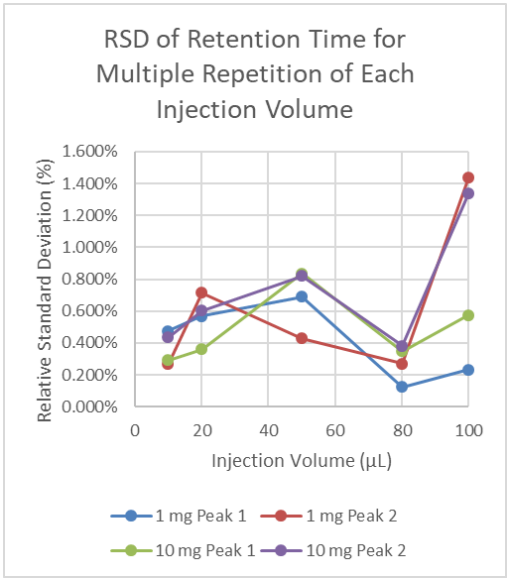


Figure 2: Relative Standard Deviation for Retention Time of Repetitive Injections to the same Volume

Injection Volume consistency is shown by evaluating the RSD of the peak integration for the repetitive injection volumes. In Figure 3 it shows RSD values less than 10% (except for 100 µL injection), with values less than 5% for injection volumes between 20 µL and 80 µL. The increased deviations outside this range on the 100 µL sample loop agree with deviation from linearity for peak integration above 50 µL shown in Figure 4.

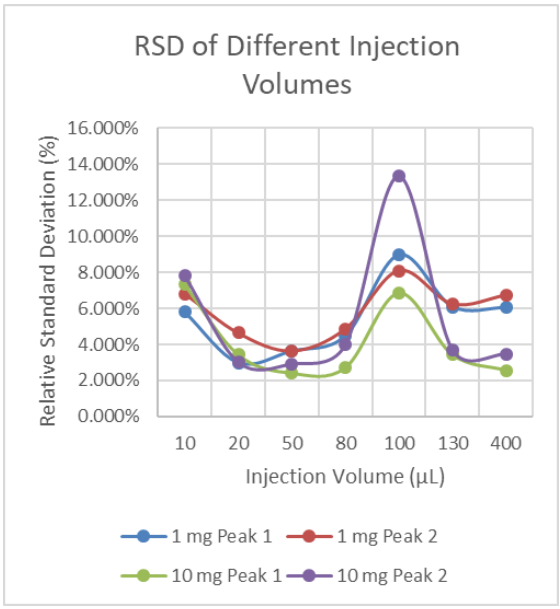


Figure 3: Relative Standard Deviation of Peak Integration of Different Injection Volumes

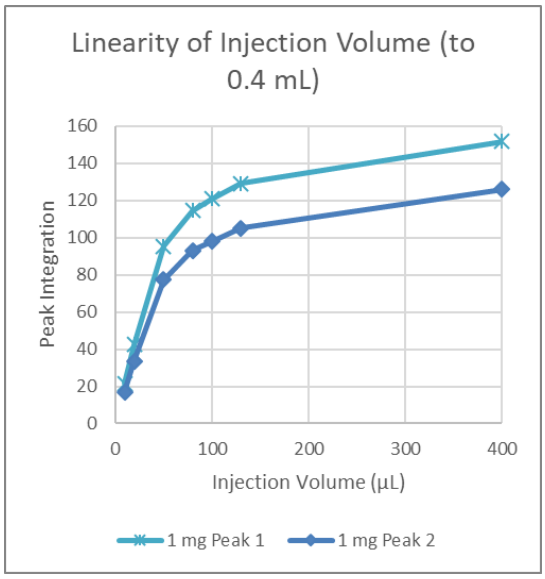


Figure 4: Linearity of Peak Integration vs. Injection Volume to 4 times loop volume (simulating a filled loop)

After peak integration, the linearity of the varying injection volumes from 10 µL to 50 µL (50% of injection loop volume) is shown in Figure 5. The R^2 values are indicative of the precision of the AutoSampler in injecting small volumes as low 10 µL. Linearity is evaluated only up to 50% sample loop volume, as sample may become lost if filled past 50% for the 100 µL loop (see Technical Note 43 *ACCQPrep® HP125 Sample Loop Maximum Injection Volume)* as observed in Figure 4.

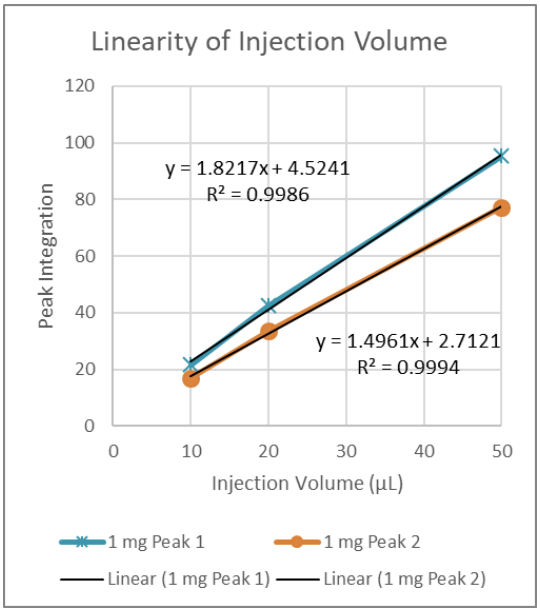


Figure 5: Linearity of Peak Integration vs. Injection Volume from 10 to 50 µL

Conclusion

The ACCQ*Prep* with AutoSampler Module can reliably reproduce injection volumes down to 10 µL on a 4.6 x 150 mm C18 Redi*Sep*® Prep Column using a 100 µL sample loop, with relative standard deviations below 5% for between 20 and 50 µL. The ACCQ*Prep* shows strong linearity of peak integration vs. injection volume size from 10 to 50 µL when using a 100 µL loop.

Successfully using sample volumes as low as 10 µL at concentrations at 1 mg/mL exhibits the ability to maximize efficiency in developing initial purification conditions for scale up using the ACCQ*Prep* while minimizing sample waste. Additionally, it exhibits the ability to use the ACCQ*Prep* to check the purity of fractions from previous separations. Using this column and sample loop size, the versatility in using the ACCQ*Prep* and AutoSampler Module for method development is demonstrated.

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