ACCQ*Prep* HP125 Sample Loop Maximum Injection Volume

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Chromatography Technical Note TN43

Overview

The ACCQP*rep* HP125 is a HPPLC (High Performance Preparative Liquid Chromatography) system. Based on the generally accepted maxim "one should not exceed one-half of the total loop volume when filling the loop partially," our maximum suggested injection volume is limited to 50% of the volume of the sample loop, resulting in the following warning:

> The programmed injection sequence will fill the injection loop more than half (50%). This may result in loss of compound.

Past literature is conflicted on the accuracy of partial loop filling methods, which would be predominant in HPPLC as a basis of minimizing loss of sample as injection waste while also minimizing the number of injections to maximize efficiency. One valve manufacturer[1,2] found at injection volumes larger than 50% of the loop size, sample began to be lost to waste due to the laminar flow profile of the injection in the loop. However, another valve manufacturer[3] showed that integrated peak area is a linear function throughout the volume of the sample loop as long as a small air space existed between the sample and the mobile phase in the loop.

Both manufacturers suggested that precision in both cases was subject to careful and consistent loading technique, something that the AutoInjector offers. Additionally, the AutoSampler is programmed to draw 50 µL of air before the sample volume in order to prevent mixing between the sample and residual mobile phase.

Reports from customers and ancillary observations, in addition to conflicting literature led us to investigate the maximum limits of sample injection volume as it relates to sample loop size.

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: 0.1 mL
Equilibration Volume: 8.1 mL
Flow Rate: 2.0 mL/min
Max Pressure: 6000 psi

Column: Redi*Sep*® Prep C18 20x150 mm
Sample Loop: 0.1 mL, 5 mL and 10 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	30
1	30
9.8	100
2.1	100
0	30
2.1	30

Detection: UV (254 nm)
Injection Method: AutoSampler (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 or AutoInjector. 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 be sure the wash reservoir is full by washing the sample probe via the AUTOMATION MANUAL CONTROL interface as shown in Figure 1.

1. Biggs, W. R. *J. Chromatogr. Sci.* 1982, *20*, 95.
2. Rheodyne Tech. Note 5 https://www.idex-hs.com/media/wysiwyg/injection-guide/tech_note_5.pdf
3. Harvey, M. C.; Stearns, S. D. *J. Chromatogr. Sci.* 1982, *20*, 487.

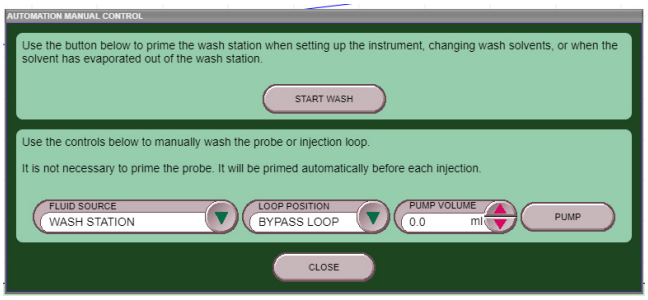

Figure 1: Automation Manual Control Screen

Data Analysis Method

PeakTrak doesn't offer a method for integration of peak area. In order to evaluate the efficiency of injection volumes the pertinent chromatographic data was extracted from the run (.txt) file as described in and integrated using a third party integration software package.

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

100 µL Loop; Redi*Sep*® Prep 4.6x150 mm C18

After peak integration, you can see the loss of linearity with injection volumes above 50% of the sample loop volume in Figure 2. A strong linearity of the varying injection volumes from 10 µL to 50 µL (50% of injection loop volume) is shown in Figure 3.

From these linear regressions, we can extrapolate what the expected peak integration area should be for the injection volume above 50% of the sample loop volume, and then determine the deviation due to sample lost during the loading process. From Figure 4, we can see the deviation from the expected area is between 20 to 40% for injection volumes between 50 to 100 µL. From this information we can assume that this would be the expected sample loss by injecting above 50 µL on a 100 µL sample loop.

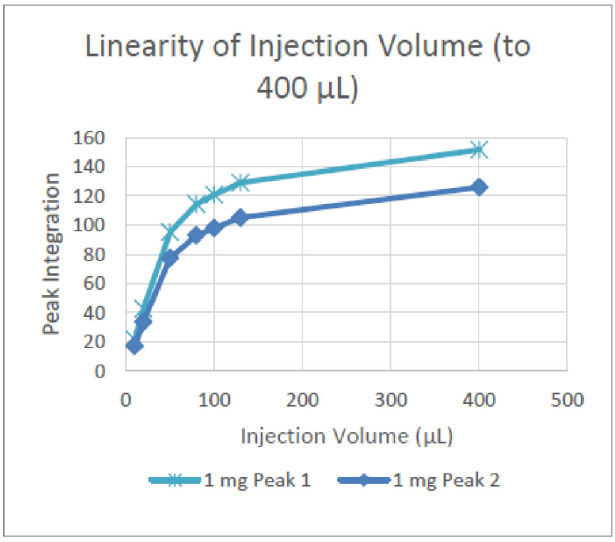

Figure 2: Peak integration across multiple, different injection volumes for 100 µL loop

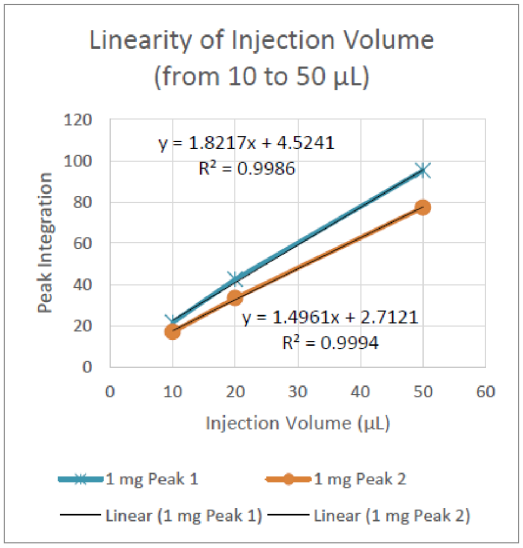

Figure 3: Linearity of peak integration from 10 µL to 50 µL on a 100 µL loop

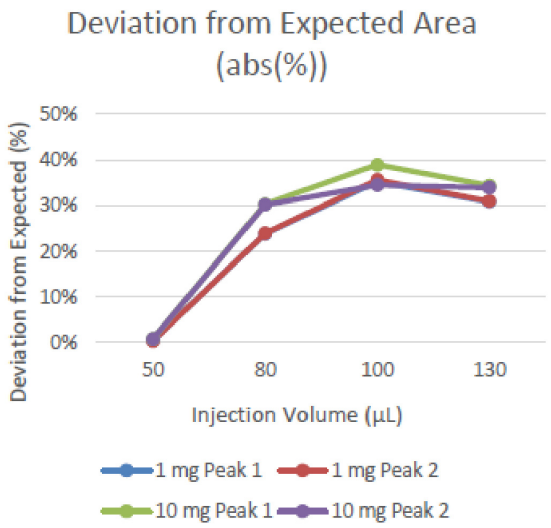

Figure 4: Deviation from expected peak integration (50 µL to 130 µL) based upon linear response from 10 µL to 50 µL for a 100 µL loop

5 & 10 mL Loop; RediSep® Prep 20x150 mm C18

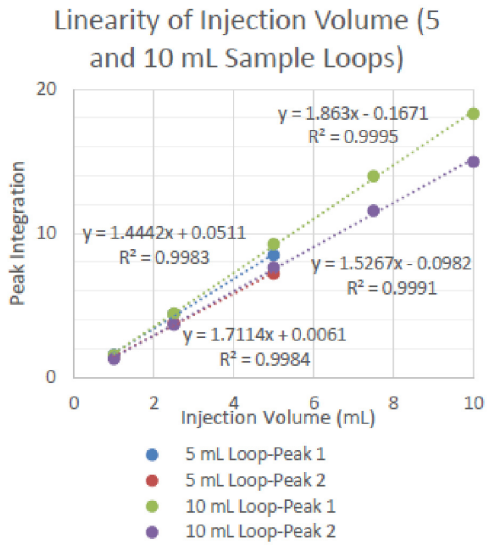

Figure 5: Linearity of Peak Integration from multiple, different injection volumes for 5 and 10 mL loops

However, for the 5 and 10 mL sample loops we observed that linearity was maintained throughout the entire range of the sample loop (1 to 5 mL for the 5 mL loop and 1 to 10 mL for the 10 mL loop) as seen in Figure 5. This means that sample loss would be negligible in these loop sizes.

We suspect that the laminar flow profile seen in the 100 µL loop (with $^1/_{16}$" OD tubing throughout) is changed upon the transition from $^1/_{16}$" to a $^1/_8$" OD tubing on the 5 and 10 mL loops.

Conclusion

We have determined the amount of sample lost depends on the size and diameter of the sample loop and the maximum injection volume. For small loops ($^1/_{16}$" OD diameter throughout) sample may be lost if more than 50% of the loop is filled. For larger loops, that transition from $^1/_{16}$" to $^1/_8$" OD tubing for the sample loop we find that 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) we suggest not filling past 50% the loop volume in order to minimize sample loss.

For larger loop sizes (5 mL and larger, we suggest a maximum injection of 1 mL less than the sample loop size. So 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.

Additionally, the reproducibility of the AutoInjector and AutoSampler methods for injection provide superior results versus manual injection as the process is more consistent from injection to injection, as the literature notes that "precision and accuracy of partially loading a loop are functions of the operator and syringe."[3]

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