Achieving Accuracy and Precision with Rheodyne Manual Sample Injectors

Abstract. The accuracy and precision of HPLC sample injection depends on injector design and loading techniques. This article describes the performance of different Rheodyne injectors and answers the following questions:

• How large a volume can be partially loaded into a sample loop with syringe accuracy?
• How much sample must be wasted to completely fill a sample loop with pure sample?
• What precision can be achieved by the various designs and loading techniques?
• How can cross-contamination be avoided?

Manual sample injectors for HPLC transfer sample at atmospheric pressure from a syringe to a sample chamber. The chamber is then connected by valving action to the high-pressure mobile phase stream that carries the sample onto the column. There are two methods of loading the sample chamber: “complete-filling” and “partial-filling.” These two techniques differ in accuracy, precision, and the amount of sample required.

Rheodyne offers several models that use only the complete-filling method. (See Table I). This method produces volumetric precision often better than 0.2% relative standard deviation. The volume injected is that of the sample loop. An excess of two to five sample loop volumes should be loaded. Sample volume is varied by changing the sample loop size.
Rheodyne Models 7125, 8125, 7725i, 9725i and 3725i-038 (see Table I) can use both the complete-filling and the partial-filling methods. With partial-filling, the volume injected is that dispensed from the syringe. Therefore, the sample volume is easily changed. The volumetric precision of partial-filling is the syringe repeatability, typically better than 1%.

Model 3725 is designed for preparative-scale liquid chromatography. Accuracy and precision are not main concerns when using preparative scale valves as sample volumes are usually larger (see Table I), and therefore, the percent relative standard deviation (%RSD) is often higher.

Table I. Characteristics of Rheodyne Manual Sample Injectors.

<table>
<thead>
<tr>
<th>Type and Capabilities</th>
<th>Scale</th>
<th>Partial-Filling Volumes (Range)</th>
<th>Sample Loop Sizes (Range)</th>
<th>Liquid-contact Materials</th>
<th>Max. MPa1</th>
<th>Max. T °C</th>
<th>MBB2</th>
<th>Model3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual Mode</td>
<td>Analytical</td>
<td>1 µL - 2.5 mL</td>
<td>2 µL - 5.0 mL</td>
<td>316 SST, Vespel4, ceramic, PEEK</td>
<td>48</td>
<td>80°</td>
<td>Yes</td>
<td>7725, 7725i</td>
</tr>
<tr>
<td></td>
<td>Micro</td>
<td>1 µL - 5.0 mL</td>
<td>2 µL - 10 mL</td>
<td>PEEK, Tefzel4, ceramic</td>
<td>34</td>
<td>50°</td>
<td>Yes</td>
<td>9725, 9725i</td>
</tr>
<tr>
<td></td>
<td>Preparative</td>
<td>0.1 µL - 500 µL</td>
<td>5 µL - 1.0 mL</td>
<td>316 SST, Vespel ceramic, PEEK</td>
<td>48</td>
<td>80°</td>
<td>No</td>
<td>8125</td>
</tr>
<tr>
<td></td>
<td>Analytical</td>
<td>100 µL - 10 mL</td>
<td>2.0 mL - 20 mL</td>
<td>316 SST, PEEK</td>
<td>34</td>
<td>50°</td>
<td>Yes</td>
<td>3725(i)-038</td>
</tr>
<tr>
<td>Single Mode</td>
<td>Analytical</td>
<td>Not Applicable</td>
<td>5 µL - 5.0 mL</td>
<td>316 SST, Vespel</td>
<td>48</td>
<td>150°</td>
<td>No</td>
<td>7010</td>
</tr>
<tr>
<td></td>
<td>Micro</td>
<td>5 µL - 10mL</td>
<td>0.5 µL - 5.0 µL</td>
<td>316 SST, Vespel</td>
<td>48</td>
<td>150°</td>
<td>No</td>
<td>7410</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 µL - 1.0 µL</td>
<td>316 SST, PEEK</td>
<td></td>
<td>48</td>
<td>80°</td>
<td>No</td>
<td>7520</td>
</tr>
</tbody>
</table>

1. This is the maximum pressure in MPa to which the valve can be adjusted. Some models are shipped from the factory set for lower pressures.
2. MBB™ (Make-Before-Break) is a patented Rheodyne design that provides uninterrupted flow when switching between LOAD and INJECT. MBB provides zero sample loss, no cross-contamination and greatly reduces transient pressure shocks.
3. Models with an "i" suffix have a built-in position sensing switch. Models 8125 and 9010 each has a built-in switch.
4. Vespel and Tefzel are trademarks of E.I. DuPont.

Rheodyne Models 7125, 8125, 7725i, 9725i and 3725i-038 (see Table I) can use both the complete-filling and the partial-filling methods. With partial-filling, the volume injected is that dispensed from the syringe. Therefore, the sample volume is easily changed. The volumetric precision of partial-filling is the syringe repeatability, typically better than 1%.

Model 3725 is designed for preparative-scale liquid chromatography. Accuracy and precision are not main concerns when using preparative scale valves as sample volumes are usually larger (see Table I), and therefore, the percent relative standard deviation (%RSD) is often higher.

This Technical Note describes the characteristics of these injectors and the loading techniques that produce the best analytical results. Some caveats: Precision values are always stated as one standard deviation or as %RSD. Keep in mind that injector precision values refer only to the injector’s reproducibility when transferring sample onto the column. The precision of peak heights and areas observed with a chromatograph will typically be worse because non-injector components also contribute to non-reproducibility. Some explanations of injector behavior are simplified, but additional discussions are contained in footnotes.

Sample chambers can be a loop of tubing or a machined passageway, but we use the term sample loop for all chambers, regardless of shape.

![Diagram of Pressure Loading](image1)

![Diagram of Suction Loading](image2)

Fig. 2. Pressure loading and suction loading with P/N 7012 and 9012 Loop Filler Ports connected to Models 7010 and 9010 injectors respectively.
Filling Characteristics

The mobile phase that flows through the sample loop in the INJECT position is trapped when the handle is returned to LOAD. As the next sample is loaded, pushing mobile phase ahead of it, the front of the sample becomes diluted. This happens because the fluid has a parabolic velocity profile between the tube walls. At the center of the tube the velocity is about twice the average, and at the wall the velocity is zero. This laminar flow effect is illustrated in Figure 3.

The initial fluid element (not shown) begins to elongate between tube walls and the sample distributes throughout a longer section of the tube, with the sample in the center having traveled farthest. The sample occupies about 2 µL of loop for every 1 µL loaded from the syringe. The exact distance traveled depends on the passageway geometry and the loading flow rate (1).

This behavior accounts for the shape of a curve that is an important injector characteristic. Figure 4 is a plot of sample mass injected onto the column (as indicated by peak height or area) vs. volume of sample dispensed from the loading syringe. This region of the plot in Figure 5 is called the Offset Volume Region. This region shows the offset (error) between the volume dispensed from the syringe and the volume that enters the loop. i) The curve is not linear because the first sample entering the loop is dilute; it is the sample traveling down the center of the connecting passage. ii) The region of complete-filling is reached later, especially when small loops are used, because the connecting passage must also be flushed.

Therefore, single mode injectors can achieve high precision only by using the complete-filling method. The following discussion shows how these filling characteristics affect accuracy, precision, and cross-contamination.

Accuracy

Often it is not necessary to know the actual volume injected onto the column, since errors are usually constant, the same during calibration and analytical runs. However, when absolute accuracy is required, the following precautions are necessary.

Partial-Filling. Partial-filling with accuracy is only practical with dual mode injectors. A safe rule is to assume that the linear region (in Figure 4) extends only to 50% of the loop volume.

Complete-Filling. A “complete loop volume” is only injected if enough sample is loaded to flush out the residual mobile phase. Table II lists the volume of sample that must be transferred from the syringe into various injectors to achieve 95% of the maximum. The actual volume contained must be determined by experimental measurement.

<table>
<thead>
<tr>
<th>Loop Volume (µL)</th>
<th>7520</th>
<th>24100</th>
<th>7010</th>
<th>7125</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2</td>
<td>3</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>30</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>40</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>55</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>95</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

These are approximate values intended to show the relative volumes required. Actual volumes will depend on operator technique (see Footnote 1).

Precision

The reproducibility of peak height and peak area in LC depends on (a) injector precision, (b) stability of flow rate, mobile phase composition, temperature, and (c) detector and integrator sensitivity fluctuations. When non-injector components are the major source of variations, different injectors, operators, or loading techniques are indistinguishable. When non-injector contributions are carefully controlled, the differences can be significant. The section on page 6 discusses non-injector contributions. The injector contributions are discussed below.

Partial-Filling. As shown in Figure 4, the syringe volume dispensed and sample mass injected are linearly related in the partial-filling region of dual mode injectors. The precision of the injector is simply that of the syringe. Consider a 25 µL syringe with fifty divisions. If the plunger can be set with a precision of 0.1 division, the result is the Syringe Volumetric %RSD shown in Table III. As the loaded volume becomes smaller, the relative error of the constant 0.1 division setability becomes larger.
Table III. Chromatograph Peak Height % RSD.

<table>
<thead>
<tr>
<th>Loaded Volume</th>
<th>Volumetric %RSD:</th>
<th>Peak Height %RSD:</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µL</td>
<td>0.25</td>
<td>0.2</td>
</tr>
<tr>
<td>10 µL</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>2 µL</td>
<td>2.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

The chromatograph Peak Height %RSD in Table III was experimentally determined using a 25 µL syringe and a 50 µL sample loop on a Model 7125 Injector.

Special equipment (see page 6) kept non-injector contributions to height and area precision below 0.05% RSD. The chromatograph values are essentially due to the injector alone. Results varied with different syringes, loops, and operators, but the values in the table are typical.

We conclude that partially loading the dual mode injectors should produce an injector precision in the range of 0.2 to 2% RSD, depending on how much of the syringe full scale is used, and on operator care.

Complete-Filling. Figure 4 shows that if enough sample is loaded the loop will contain almost pure sample. The zero slope of the line eliminates the syringe as a factor. The volumetric precision should be good because of the mechanical stability of the sample loop. The contained sample mass precision will then be governed by the stability of sample density. Table IV shows the precision that results from a density coefficient of 0.1% per °C (3).

Table IV. Volumetric Precision Based on Density Coefficient.

<table>
<thead>
<tr>
<th>Temperature Stability</th>
<th>%RSD of Injected Mass</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
</tr>
</thead>
</table>

Using the special equipment, we loaded 350 µL of sample into a 5 µL loop on a dual mode injector, thermostated at ±0.1°C. The observed chromatograph peak height precision was 0.03% RSD, so we can state that the injector was at least this good (see page 6).

Figure 4 shows that smaller load volumes will produce poorer precision. The slope increases as less sample is loaded, resulting in an increasing contribution by the syringe non-reproducibility. Our experiments show that in the load-volume range of roughly half to two loop volumes, the precision is often even worse than that of the syringe itself (4). So if enough sample is available, it is good practice to load with more than two loop volumes. An injector precision of about 0.1% RSD is typical when a dual mode injector is loaded with about five loop volumes.

This behavior is representative of loops in the 5 µL to 200 µL range on most Rheodyne injectors. However, injectors with smaller chambers – Models 7410 and 7520 – provide less precision, even when a large excess of sample is used. Values are typically in the 0.2 to 2% range, when loaded with 95% of the maximum sample mass (Table II). This difference from larger volume injectors is apparently due to operator skill and subtle variations in manual loading. The dimensional stability of the micro-loops is good, as indicated by the 0.05% RSD observed when loaded under automatic control (5).
Cross-Contamination

Mobile phase automatically flushes out the sample loop while in the INJECT position, unless the injector is returned to LOAD prematurely (6). However, the connecting passage between needle and loop still contains sample and must be flushed before every injection. Small, well swept passages, require less flushing solvent. Rheodyne’s dual mode injectors are special cases because they have no connecting passage. Even though it has been designed to eliminate the trapped volume, we would expect there to be a very small amount of cross contamination if the injector is not flushed between injections. This is because of sample traces left on the surface of the needle seal and rotor seal, and in the liquid left in the needle port. We have made many direct measurements of this by injecting a blank after a normal sample, without an intermediate flush. Figure 6 shows typical results. The carryover is only observable when the sample concentration is very high and the detector is operated at a sensitive range.

We found that the amount of contamination varies with conditions, and even from one particular injector to the other. For example, with water as mobile phase and sample solvent, the cross contamination was 0.004 µL, but with heptane as mobile phase and sample solvent the cross contamination was 0.001 µL. Contamination was always less than 0.01 µL, which represents less than 0.1% for a 10 µL injection. Users of the 7125, 7725, and 8125 who want to use the valves without flushing should actually check the cross contamination under the particular conditions at hand if there is any concern about its magnitude. The peak height that results from the sample should not exceed the linear range of the detector, or the cross contamination will be overstated. It is good practice to flush the 7125, 7725, and 8125 every five or ten injections to prevent the possible build-up of contaminants and to keep both needle port and vent line full of solvent (7).

Summary

The partial-filling method uses syringe readings to determine the injected volume of sample. These readings are accurate only within the linear region of the load volume curve. With this filling method, linearity extends from zero to about half a loop volume since there is no internal connecting passage. Precision with partial-filling is about 1% RSD. It depends on the syringe and operator technique. With Models 7125 and 7725, when 5 µL is dispensed with care from a 10 µL syringe into a loop 10 µL or larger, a precision of injected sample mass of about 0.4% RSD will result. The observed precision of peak height and area will be larger due to non-injector chromatograph components.

Cross-contamination can be avoided by flushing needle ports properly. Dual mode injectors can often be used without flushing after each injection, but it is good practice to flush after every few injections.
Footnotes

(1) The dispersion of sample in straight tubular passages is flow rate dependent. At practical rates of loading, the interaction of diffusion with the parabolic velocity profile increases to increase with the flow rate. Sample dispersed more quickly from a syringe will travel further along the passages. This flow dependence can contribute significantly to loss of accuracy. A precision sample loop constant loading technique. The following factors depend on the loading flow rate: (a) The offset volume, the point at which sample enters the loop from a connecting passage, (b) the volume at which the linear region is exceeded, and (c) the volume at which the loop is filled with a specific percentage of its maximum contents.

When passages are short, curved, or change diameter abruptly, the dispersion mechanism becomes complex. The dispersion flow dependence can be quite different from that described above. The practical consequences are the same: injection behavior depends on the rate at which sample is loaded into the loop.

All discussions in this Technical Note assume that the loop always contains mobile phase prior to loading and that the sample is introduced without air bubbles. However, two other techniques are sometimes used. 1) The mobile phase is displaced by air before the sample is loaded. The amount of sample required to flush the sample loop completely is reduced, since there is nothing to dilute the sample. 2) Three or four small segments of air are placed in front of the sample. These segments reduce mixing as the sample is inserted. They also cause an increase in retention time of about 10% for full injection, but there is no significant loss of sample.

Different solvents or air, artifact peaks may be produced, especially at high detector sensitivity and low UV wavelength. These problems are avoided by flushing the needle port with mobile phase after each injection, or at least frequently enough to keep the vent line and needle port full. A Needle Port Cleaner is available and can be used for this purpose. Further, this flushes the entire length of the port.

Models 7125, 7225, and 8125 are most efficiently flushed while in the INJECT position, since the flush solvent exits via a vent line and does not pass through the sample loop. Flushing in the LOAD position fills the sample loop with flush solvent. This should cause no problem with the complete-filling method since most or all of it will be displaced by the sample. But with partial-filling some of the flush solvent will become injected. Unlike the complete-filling method, the mobile phase (trace contaminants, oxygen content, etc.) may cause artifact peaks.

Dependence of Analytical Precision on Variations in Flow Rate, Composition, Temperature, and Experimental Methods.

The reproducibility of peak area and peak height depends on the stability of flow rate, mobile phase composition, column temperature, and the precision of the injector. This section summarizes the relative importance of the non-injector variables. It applies only to concentration-sensitive detectors (UV, refractive index and to some extent electrochemical).


Flow Rate. Peak area is inversely proportional to flow rate; a 1% flow rate decrease causes a 1% area increase. Peak height is somewhat immune to flow changes, the dependence being due to the effect of flow rate on column efficiency. A 1% flow rate decrease usually causes <0.3% peak height increase. However, height can be affected when the time constant of the detector/data system is slow compared to the speed of peaks.

Mobile Phase Composition. Peak area is relatively unaffected by changes in composition. A decrease in B modifier causes an increase in peak width and a corresponding decrease in peak height. The area is unchanged. The different composition can change detector response, but this effect is usually small.

Peak height is very dependent on mobile phase. A 1% (absolute) decrease in B modifier causes height decreases in the range of 1 to 10%, depending on the mobile phase and capacity factor of the peak.

Temperature. Peak area is only slightly affected by temperature changes, the mechanism being the temperature dependence of detector response. Peak height is affected because of the temperature dependence of retention and column efficiency. The dependence varies widely among different compounds and mobile phases. A decrease of one degree Celsius typically causes an increase in retention time of about 2%, and a corresponding decrease in peak height. Solutes with secondary equilibria, such as acidic or basic compounds can be much more sensitive to temperature changes.

Table V summarizes how peak height and area typically change with a small incremental decrease in flow rate, composition and temperature. It shows that flow rate control is most important when area is used to quantify, while composition control is most important when height is used.

Table V. Variations in Conditions Affecting Peak Area and Height.

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>Composition</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area +1%</td>
<td>+1%</td>
<td>-1°C</td>
</tr>
<tr>
<td>Height +0.3%</td>
<td>-1 to +1%</td>
<td>0 to +2%</td>
</tr>
</tbody>
</table>

Experimental Methods. In the experimental work, non-injector contributions to total chromatograph precision were suppressed in order to make the differences between injectors more apparent. The chromatograph was modified to reduce the random flow fluctuations to <±0.1% and the cyclic noise to <±0.5%. Composition variance was eliminated by using pure water or a premixed binary solvent as mobile phase. Two types of columns were used: packed columns operated at about 3000 psi, and inert open capillary tubes that produce a single peak. Column temperature was controlled to <±0.1°C.

By these means the precision of the total chromatograph (injector plus non-injector contributions) was 0.05% RSD for peak area and 0.03% RSD for peak height, for the best case injector. Flow rate fluctuations are probably responsible for the larger area value, since area is more flow dependent, and since the composition and temperature variations were very small. (When flow rate was allowed to drift, the area %RSD changed much more than the peak height %RSD). Therefore, we used peak height precision as the most accurate indicator of injector precision. %RSD values an order of magnitude larger should be expected with most chromatographs.