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Achieving Accuracy and Precision with Rheodyne 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?*

Modern 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, which carries the sample into 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 Models 7010, 7410, 7413 and 7520 use the complete filling method. This produces volumetric precision often better

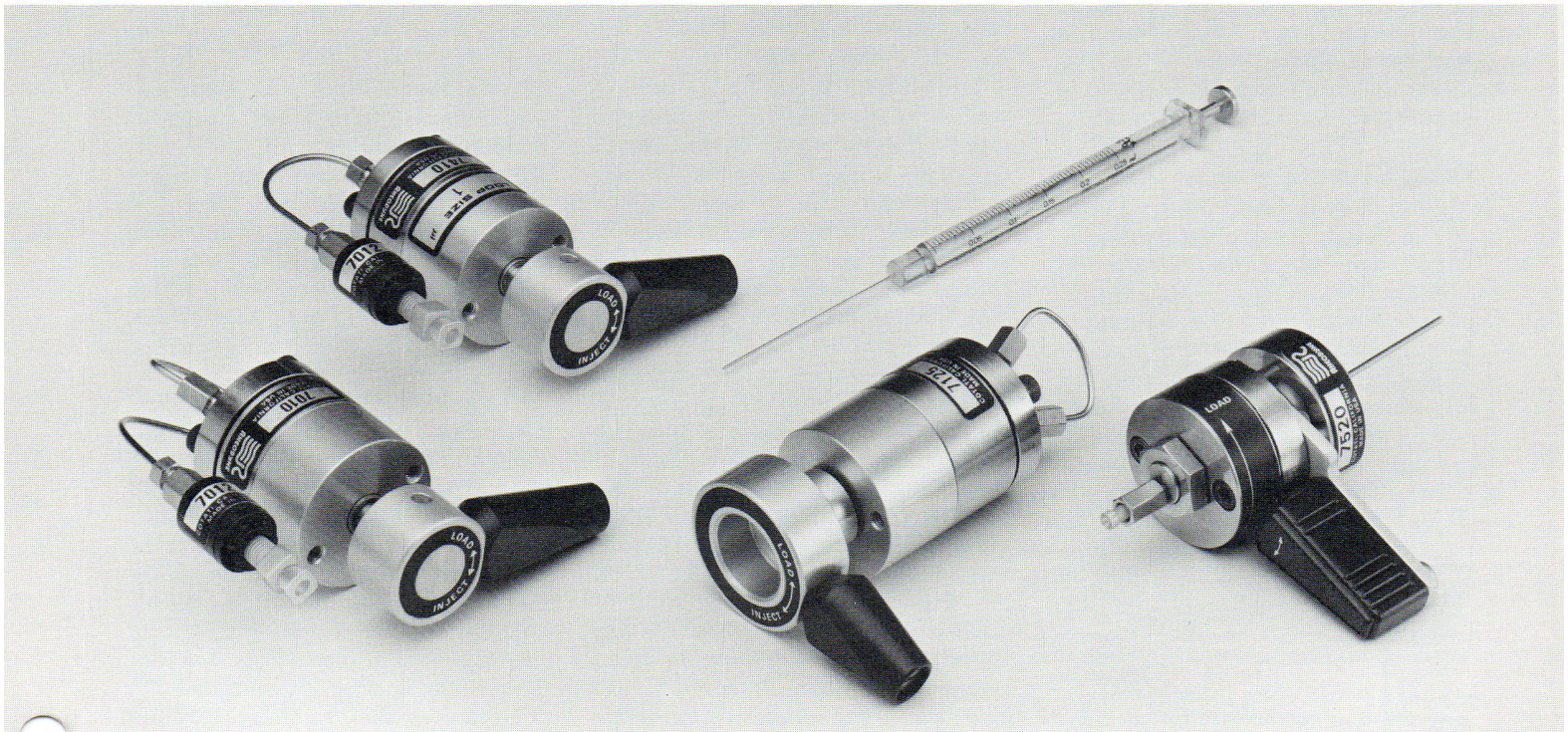


Figure 1. Four Rheodyne sample injectors. Models 7010 and 7410 use the complete filling technique. The sample loop is loaded via an external connecting passage, such as the Model 7012 Loop Filler Port accessory, shown here attached to both injectors. The 7010 has external loops ⩾5 μl, the 7410 has internal loops ⩽5 μl. Model 7520 also uses complete filling, but has a built-in needle port for loading the internal ⩽1 μl sample chambers. The Model 7125 can use both complete and partial loading. In partial loading, all of the sample dispensed from the syringe enters the sample loop. This zero-sample-loss feature is unique among injectors that can use both loading techniques. Sample volumes range from <1 μl to 5 mL. The needle port is built into the center of the handle, but is not visible in this photograph. The Model 7126 (not shown) is a pneumatically actuated version of the 7125.

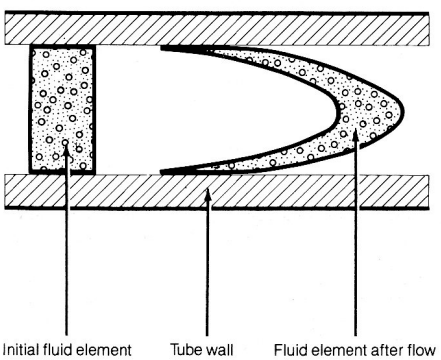
than 0.2% relative standard deviation. The volume injected is that of the chamber, an excess of sample being used to load it. Sample volume is varied by changing the sample chamber size.

Rheodyne Models 7125 and 7126 can use *both* the complete filling and the partial filling method. With partial filling, the volume injected is that dispensed from the syringe, so sample volume is easily changed. The volumetric precision of partial filling is the syringe repeatability, typically better than 1%.

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 percent relative standard deviation (%RSD). Keep in mind that injector precision values refer only to the injector's ability reproducibly to transfer sample into the column. The precision of peak heights and areas observed with a chromatograph will be worse than this, because non-injector components also contribute to the scatter of data. Some explanations of injector behavior are simplified; 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.

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 across the tube. At the center of the tube the velocity is about twice the average, and at the wall it is zero. This laminar flow behavior is illustrated below.



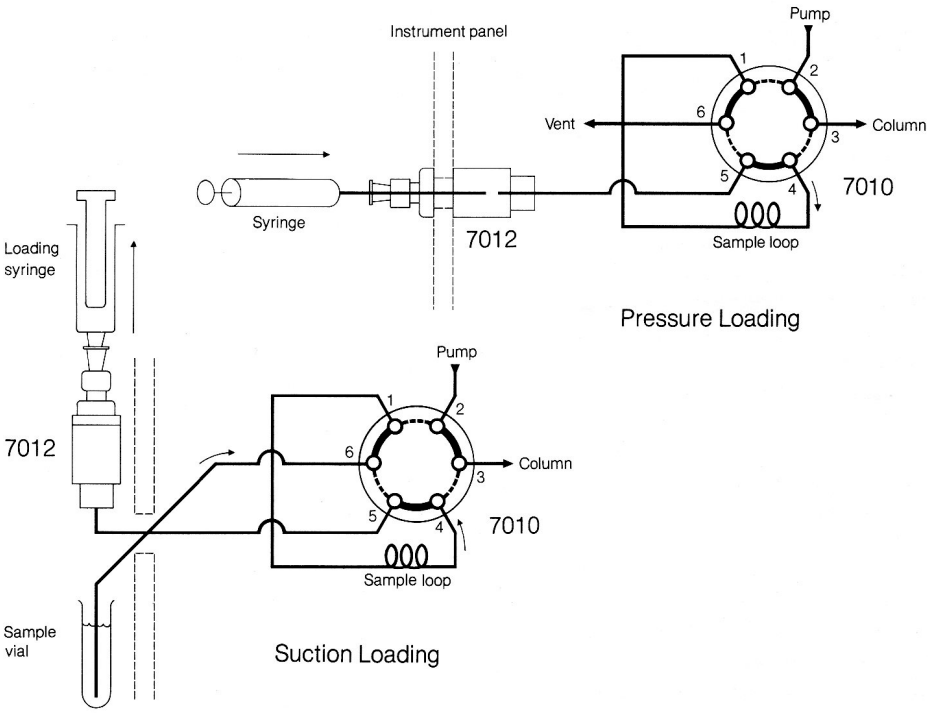


Figure 2. Model 7012 Loop Filler Port connected to a Model 7010 Injector in two ways. In pressure loading, sample is dispensed from a needle. It requires a minimum amount of sample because the 0.3 mm (0.012 inch) I.D. connecting tube contains only about 7 µl. Sample volume requirements can be further reduced by using smaller diameter tubing. In suction loading, sample is sucked from a vial into the loop via a dip tube attached to the injector. There is no syringe to flush out after each injection, the dip tube is easily wiped or rinsed off, and the sucking syringe can be used for many injections before it needs emptying.

The initial fluid element represents a small segment of the sample's leading edge. After flow the solute is distributed 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. The curve is a plot of sample mass injected into the column (as indicated by peak height or area) vs. volume of sample dispensed from the loading syringe. Figure 3, a plot for a Model 7010 injector with a 5 µl loop and used with a 7012 Loop Filler Port, shows three regions.

Offset Volume Region. Most injectors have a passage that connects the tip of the syringe needle to the end of the sample loop. This can be an external connecting tube, such as the Model 7012 Loop Filler Port shown in Figures 1 and 2. Or it can be an internal passage, as in the Model 7520 Syringe Loading Micro Sample Injector, Figure 1. Since sample must flow through this passage to reach the loop, there is an offset (error) between the volume dispensed from the syringe and the volume that enters the loop.

Partial Filling (linear) Region. The offset causes the initial rise of the curve to be non linear. When the offset volume is small compared to the loop volume, there is a substantial portion of the curve which is effectively linear. The curve becomes non linear again at about 50% to 70% of the loop volume.

Complete Filling (nonlinear) Region. After sample reaches the far end of the loop, the mass of sample contained increases nonlinearly as additional sample is loaded. It asymptotically approaches a maximum value, which is when the entire loop contains pure sample, undiluted by residual mobile phase. A convenient, but arbitrary, point for comparing injectors is the loaded volume at which the loop contains 95% of the maximum sample mass.

Figure 4 is a plot for a Model 7125 injector with a 20 µl loop. Note the following differences from Figure 3. The offset volume is zero, and the curve is linear starting at zero volume. This is because of a unique feature. There is no needle-to-loop connecting passage; the needle directly abuts

the end of the loop. Syringe readings are accurate even for volumes less than 1 μl. The loop contains 95% of the maximum sample mass after about 40 μl have been loaded.

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 into 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. In the linear region of the curve, the offset volume must be subtracted from the syringe reading. A safe rule is to assume that the linear region extends only to 50% of the loop volume. Partial filling with accuracy is only practical with Models 7125 and 7126.

Complete Filling. A "complete loop volume" is only injected if enough sample is loaded to flush out the residual mobile phase. Table I 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 (2). This is because stated volumes on loops are nominal (errors can be as high as 30% in small loops). Also, in the case of external loops, the same loop

Table I: Approximate Load Volumes in μl for 95% of Maximum Sample Mass(1)

Loop Volume (μl)	7520	7410(2)	7010(2)	7125
0.2	3			
0.5	4	40		
1	7	25		
2		25		
5		30	35	15
10			40	25
20			55	40
50			95	80

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

(2) The 7410 and 7010 data applies when using a Model 7012 Loop Filler Port with the standard (0.3 mm I.D.) connecting tube containing about 7 μl. Fill volumes can be reduced by using a tube with smaller diameter.

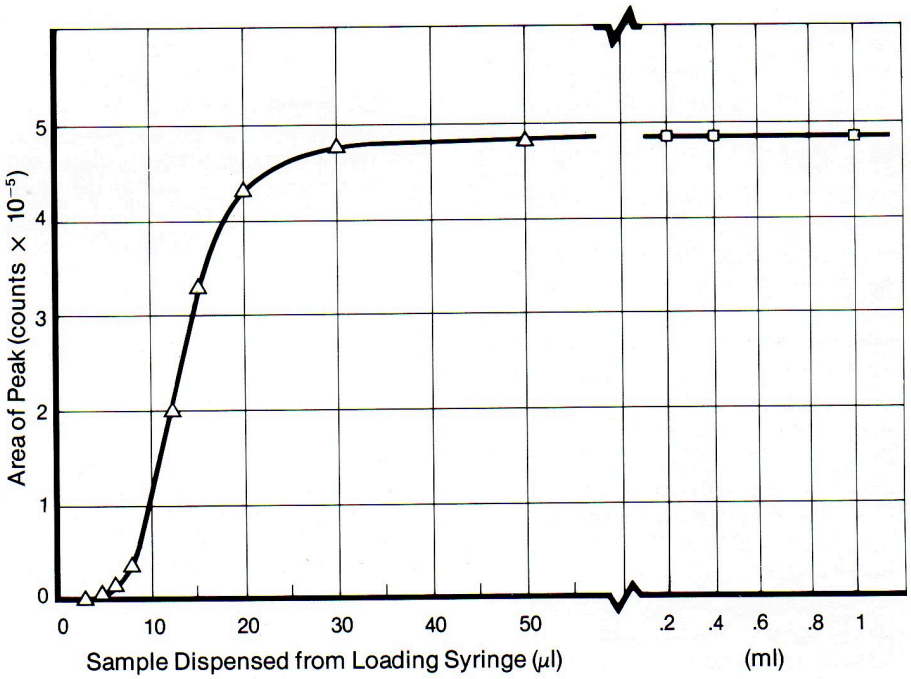


Figure 3. Plot of sample mass injected into the column vs. the volume of sample dispensed from the loading syringe, using a Model 7010 injector with a 5 μl sample loop and a Model 7012 Loop Filler Port (0.3 mm I.D. connecting tube). The first 5 μl of sample loaded does not become injected, because it has not yet reached the sample loop. After 5 μl the sample starts to enter the loop. After about 8 μl a nearly linear region is reached. At about 15 μl some sample has reached the far end of the loop and the contained mass increases nonlinearly. At about 30 μl the loop contains 95% of the maximum. After 200 μl the addition of sample causes very little increase in mass contained in the loop.

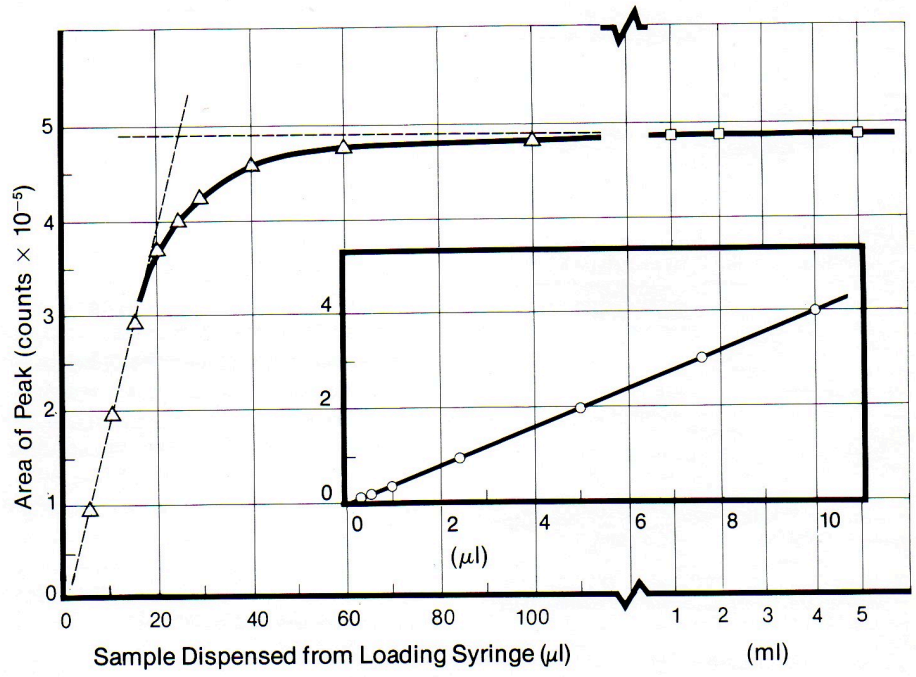


Figure 4. Plot of the sample mass injected into the column vs. the volume of sample dispensed from the loading syringe using a Model 7125 with 20 μl sample loop. Data were obtained using three syringe sizes: 10 μl (0), 100 μl (△), and 5 mL (□). The linear regression straight line best fit to the 10 μl syringe data is shown (---). The straight line correlation coefficient is 1.0000. Departure from linearity starts around 15 μl, i.e., at about 60% of the actual loop volume. The injector was flushed (INJECT position) with 0.5 mL of solvent after each injection.

on different injector models will inject different volumes. This is because the volume includes internal injector passages, the volumes of which differ among models.

Precision

The reproducibility of peak height and peak area in LC depends on (a) injector precision, (b) stability of flow rate, mobile phase composition, and 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. But 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 Models 7125 and 7126. So 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 %RSD shown below results. As the loaded volume becomes smaller, the relative error of the constant 0.1 division setability becomes larger.

loaded volume:	20 μl	10 μl	2 μl
syringe volumetric %RSD:	.25	.5	2.5
peak height %RSD:	.2	.3	1.5

The chromatograph peak height precision in the table 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. So 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 Models 7125 and 7126 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. The table below shows the precision that results from a density coefficient of 0.1% per °C (3).

temperature stability:	0.1°C	0.5°C	1°C
%RSD of injected mass:	.01	.05	.1

Using the special equipment, we loaded 350 μl of sample into a 5 μl loop on a Model 7125 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 vol-

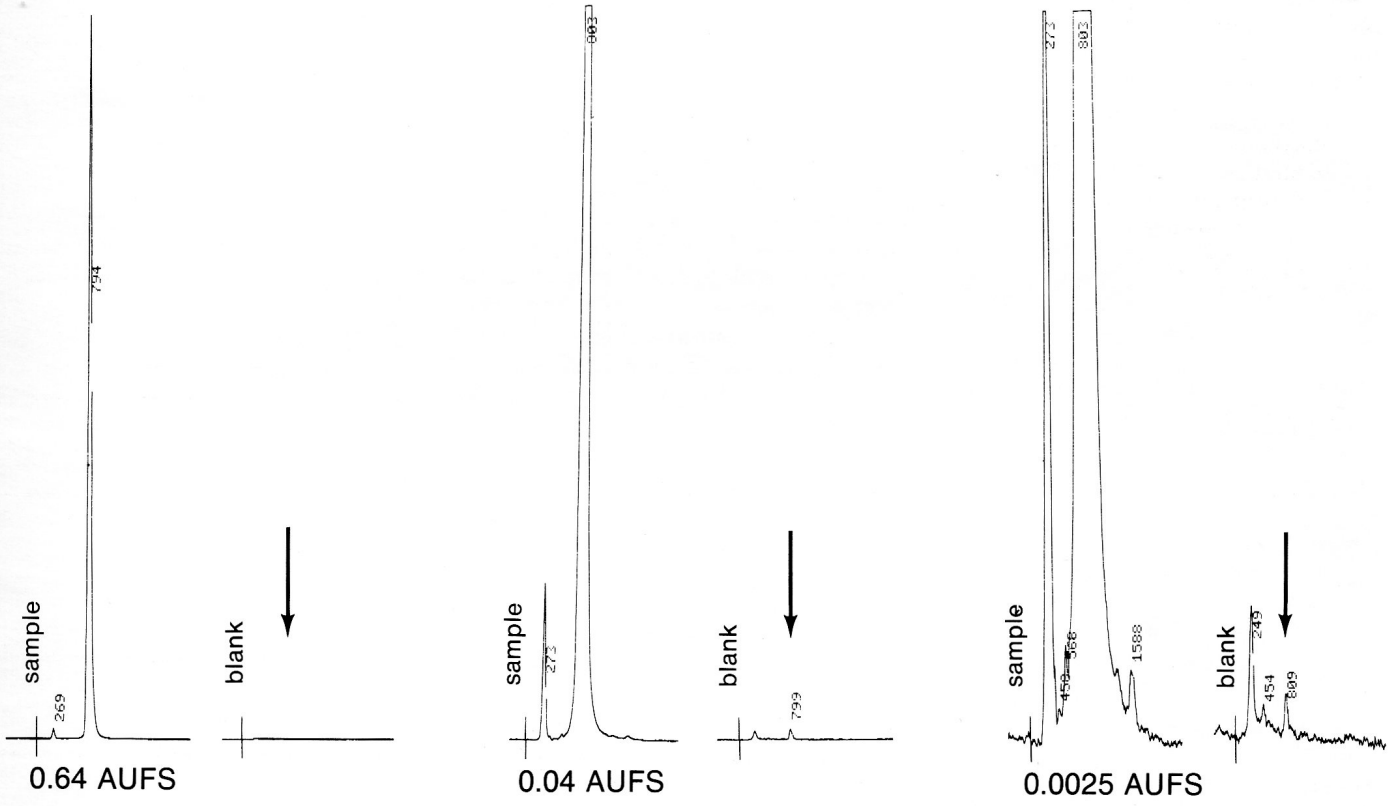


Figure 5. These chromatograms show the Model 7125's small cross contamination from one sample to the next when the needle port is *not* flushed between injections. (Sample carryover can be completely eliminated by flushing.) A 10 μl syringe injection (into a 20 μl loop) of concentrated sample was followed by a 10 μl injection of mobile phase (blank), without an intermediate flush of the needle port. This was done at three different detector sensitivities. The units of retention time printed out on the peaks are 0.1 seconds. Thus the retention time of the major sample component is 80 seconds (varies from 79.4 to 80.9 seconds in this series of runs). In the experiment at 0.64 AUFS the cross contamination cannot be observed. When the sensitivity is increased to 0.04 AUFS the small cross contamination can be seen. At a sensitivity of 0.0025 AUFS the contamination can be measured, 0.02% or 2 nanoliters absolute. At this high sensitivity, contaminants in the blank are observable. Conditions: 4.6 mm × 10 cm Hypersil ODS 5 μm column; water-acetonitrile, 1:1 mobile phase; 2 mL/min flow rate; 40°C temperature; 254 nm detector wavelength.

umes will produce poorer precision. The slope increases as less sample is loaded, resulting in an increasing contribution by the syringe nonreproducibility. 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 Model 7125 is loaded with about five loop volumes.

This behavior is representative of loops in the 5 µl to 200 µl range on Model 7125, 7126 and 7010 injectors. However, injectors with smaller chambers - Models 7410, 7413 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 I). This difference from larger volume injectors is apparently due to subtle variations in manual loading. The dimensional stability of the microloops 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.

The Model 7125 is a special case because it has 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 5 shows typical results. The carryover is only observable when the sample concentration is very high and the detector is operated on a sensitive range.

We found that the amount of contamination varies with conditions, and even from one particular injector to another. 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 contaminaion 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 who want to use it 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 every five or ten injections to prevent the possible buildup 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 the Model 7125, linearity extends from zero to about half a loop volume, since there is no internal connecting passage.

Precision with partial filling is about 0.2% to 2% RSD. It depends on the syringe and operator technique. With the Model 7125, 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.

The complete filling method uses the sample loop to determine the injected volume. When the volume must be known exactly, it must be experimentally determined. Excess sample is required to fill the loop completely with undiluted sample. With the Model 7125, about 15 µl is required to fill a 5 µl loop with 95% of the maximum contained sample mass.

Precision with complete filling is about 0.03% to 2% RSD. Micro sample loops tend toward the higher value. With the Model 7125, when 25 µl is dispensed into a 5 µl loop, a precision of injected sample mass of about 0.1% RSD will result. The observed peak precision will be less due to non-injector components.

Cross contamination can be avoided by flushing needle ports properly. The Model 7125 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 causes dispersion to increase with increased flow rate. Sample dispensed more quickly from a syringe will travel further along the passages. This flow dependence can contribute significantly to loss of accuracy and precision, unless the operator uses consistent 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. But the practical consequences are the same; injector 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 load the loop completely is reduced, since there is nothing to dilute the sample. (2) Three or four small segments of air are placed in the front of the sample. These segments reduce mixing as the sample passes through the loop, so less sample is required to fill a given volume. This is not recommended for partial filling because the air will be injected and can cause artifact peaks at high detector sensitivity. With complete filling the segments must be completely removed from the loop to insure high precision. When suction loading is used (Figure 2), the air segmentation is achieved by withdrawing the dip tube from the sample momentarily a few times as the sample starts into the tube.

(2) The actual volume injected into the column by the complete filling technique includes internal valve passages. The injected volume can be experimentally determined as follows. Plot peak height or area vs. loaded volume, as in Figure 4. Be sure all points are within the linear range of the detector. Extrapolate the ascending linear part of the curve upward, and the horizontal part to the left. At the intersection of these two extrapolated lines, drop a perpendicular to the volume axis. The volume thus found is that actually injected.

When there is an offset volume, as in Figure 3, the offset must be subtracted to determine the correct volume. The offset volume is determined by extrapolating the ascending linear part of the curve down to the volume axis.

(3) Density changes per degree centigrade are about 0.02% for water, 0.1% for most organic solvents, and intermediate values for aqueous-organic mixtures. These density changes are considerably larger than the changes in contained volume of a sample loop due to thermal expansion. For example, steel has a cubic coefficient of expansion of about 0.004% per degree centigrade. Therefore it is fluid density changes that govern the precision of sample mass injected from a completely filled sample loop. However, the presence of gas bubbles can cause very poor precision.

(4) In the complete filling method, a point is reached during dispensing of the sample, when the front of the sample reaches the end of the loop (beginning of the nonlinear region, Figure 4). As more sample is dispensed, some of it passes out of the loop into the vent line, where it cannot become injected into the column. For a given volume dispensed, the amount of sample lost from the loop depends on the flow rate of loading. This is because the distance that the sample travels down the loop

depends on the dispensing flow rate, as discussed in footnote (1).

As the load volume becomes large, in excess of two loop volumes, the sensitivity of this effect to flow variations diminishes. In the limiting case of very large load volumes, the loop is essentially 100% full of sample (no residual mobile phase), and the sample mass contained in the loop is immune to loading flow rate changes.

(5) The Model 7520 with a 1 μl sample loop produced excellent peak height and area precision even with small sample load volumes, when loaded under automatic control. This control consisted of the following: a needle was fastened to the needle port, and connected via a Teflon tube to a helium-pressurized sample reservoir. An automatic on-off valve was placed in this line to control the time during which sample could flow, and thus the total volume of sample loaded. The time duration was computer-controlled. Volumes of 6 μl and more could be loaded with .05% RSD. Volumes of 3 μl produced .2% RSD, which still compares favorably with the precision achieved by hand loading this volume. Factors which may play a role in the exceptional precision achieved under automatic control include: a) constant sample delivery flow rate, see footnote (4), b) constant orientation of the needle in the port, leading to constant sample transfer characteristics (convective mixing, etc.), c) absence of micro bubbles that may enter the needle port during insertion and withdrawal of the needle in hand operation.

(6) In the INJECT position the sample is flushed out of the loop and into the column. But, just as it requires several loop volumes to displace all the mobile phase when loading, several loop volumes of mobile phase must pass through the loop to flush out all of the sample. The number of volumes passing through the loop per unit time is simply: (mL/min) ÷ (mL/loop) = loops/min. For example, a 10 μl loop in a system running at 1 mL/min turns over 100 loop volumes per minute. A 0.2 μl loop at 10 μl/min has 50 volumes per minute. Leaving the handle in INJECT for 30 seconds will therefore flush the sample loop adquately under most conditions.

(7) The loading syringe is removed from the Model 7125 needle port in the INJECT position. As the needle withdraws it sucks about 1 μl of whatever is in the vent line into the needle seal. This material is subsequently pushed into the sample loop when the next sample is loaded, unless the needle port is flushed prior to returning to LOAD. In the partial loading method the material becomes injected into the column. If the material is mobile phase, no artifacts are produced. But if the material is a different solvent or air, artifact peaks may be produced, especially at high detector sensitivity and low UV wavelengths. 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", not a needle, should be used for flushing since this flushes the entire length of the port.

The Model 7125 is most efficiently flushed while in INJECT, 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. Unless the flush solvent is exactly like the mobile phase (trace contaminants, oxygen content, etc.) it may cause artifact peaks.

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

The reproducibility of peak area and peak height depends on the stability of flow rate, mobile phase composition, and column temperature, as well as 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). Reference: "Variables Affecting Precision and Accuracy in HPLC," S. R. Bakalyar and R. A. Henry, J. Chromatogr. *126,* 327 (1976).

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.

The table below summarizes how peak height and area 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.

	flow rate −1%	composit. −1%B	temp. −1°C
area	+1%	0 to ±1%	0 to ±1%
height	<+0.3%	−1 to −10%	0 to ±1%

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 which 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.

Measured % RSDs are only an estimate of the true standard deviation. As the number of measurements increases, there is a narrower range within which we can expect the true σ to be. For example, for an estimate of the standard deviation found to be .05%, the table below shows the range within which true σ will be found, with a confidence level of 90%. All our measurements of % RSD were made from a set of 20 runs.

Measurements	Range for true σ
5	.032 – .119
10	.036 – .082
20	.040 – .069
60	.044 – .059

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