Spark HOLLAND

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The comparison of LC autosampler injection designs

Fixed Loop versus Split Loop

Better Sample Care.

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1. Overview

Over the years, the autosampler has proven to be an essential part in an HPLC-system. An autosampler is responsible for injecting a precise volume of sample onto the column under high-pressure conditions. While all autosamplers consist of the same key-components such as an injection valve and a sample needle, there are differences in the injection principles between various autosamplers. Nowadays, two main injection designs are commonly applied: Fixed Loop designs and Split Loop designs.

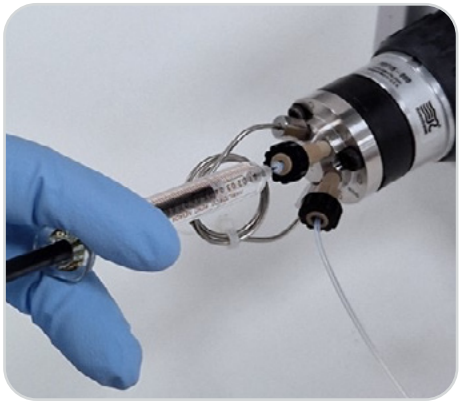


Figure 1: A manual injection

Figure 2: An autosampler

The first Fixed Loop injections in modern HPLC were performed manually, using a syringe directly dispensing the sample into the loop (figure 1). This sample loop would be overfilled to ensure that the entire loop was saturated with sample. In the early 70's, the first autosamplers were developed, which were based on an automated version of the manual Fixed Loop injection.

With the use of autosamplers, sample injection performance improved, as the sample injection was no longer prone to human error. Development in autosampler technologies, such as the use of syringe drives, resulted in optimized injection principles. The most common injection principles for a Fixed Loop design are:

- *Full Loop*
- *Partial Loopfill*
- *Microliter Pickup*

With the development of a Split Loop design, a new concept in sample injection was introduced. With this design, the needle is integrated with the sample loop. A metering device or a syringe pulls the sample directly into the sample loop, eliminating sample loss. This injection design is therefore very useful for small injection volumes or with limited sample availability.

Nowadays, nearly every modern HPLC-system relies on an autosampler, but the choice of which injection principle is the best for your application can be challenging. This paper provides insight in the comparison of the Fixed Loop and Split Loop injection designs and helps to make a considered decision for your application.

Performance characteristics

There are different characteristics that define a good autosampler. Besides requirements such as ability to withstand pressure and compatibility to a wide range of solvents, an autosampler is primarily required to obtain reproducible results. The injection principle of the autosampler can have an effect on the analytical performance of an autosampler. This section defines the performance characteristics that are tested during hands-on experiments for all of the injection principles.

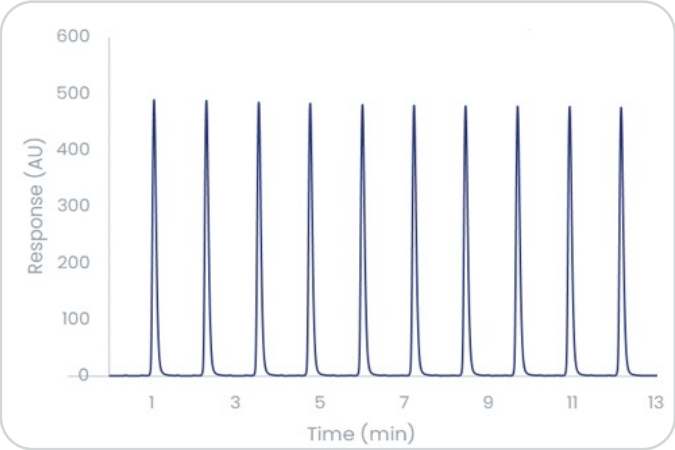


Figure 3: Reproducibility chromatogram

Reproducibility

The key aspect to measure performance of an autosampler is the reproducibility of the peak-area. The reproducibility, in this case, means the precision of the injection volume under the same circumstances. The peak area should remain the same with each injection if the same amount of the same sample is injected every time. The reproducibility will be expressed by calculating the relative standard deviation (RSD) of the peak area. A lower RSD indicates a more precise injection volume.

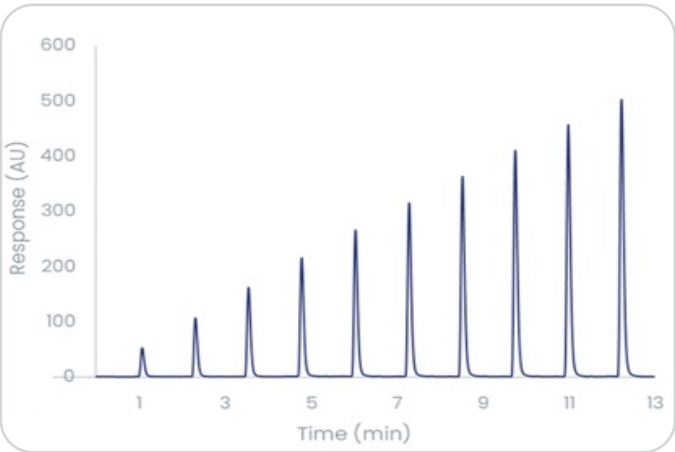


Figure 4: Linearity chromatogram

Linearity

Because a typical HPLC detector has a limited linear detection range, it is important that the autosampler injects the sample into the flow-path linearly. This means that there must exist a linear correlation between the amount of sample injected and the response of the detector; when twice as much sample is injected, the response should also be twice as high. The linearity of an autosampler can be measured by injecting the same sample, with increasing injection volumes. The linearity will be expressed using the correlation coefficient, or r^2.

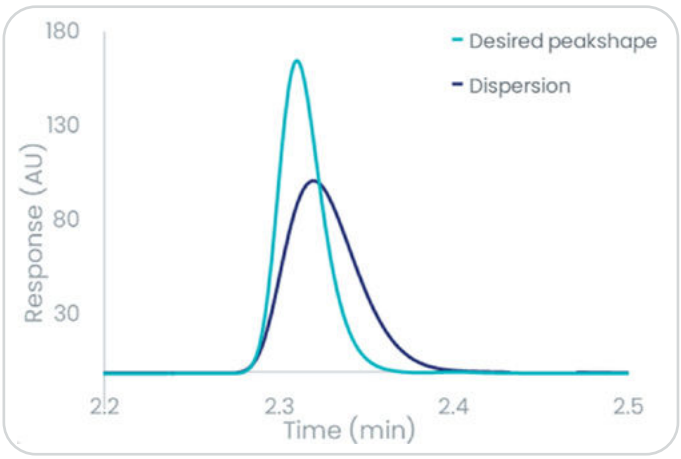


Figure 5: Example of peak dispersion

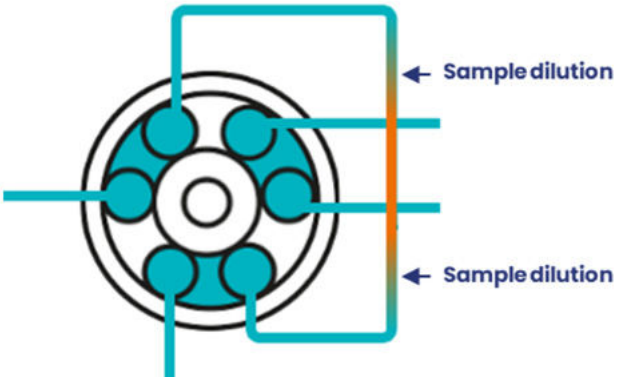


Figure 6: Schematic representation of dispersion in a sample loop

Dispersion

Dispersion can be defined as the amount of sample plug broadening between the injector and detector. This will have a negative effect on peak resolution. An example of what dispersion could look like in a chromatogram is shown in figure 5. Dispersion mainly happens because of a phenomenon called laminar flow. When a liquid is moving down a cylindrical tube, such as HPLC tubing, friction causes the fluid at the borders of the tube to flow slower than in the middle, resulting in a parabolic distribution. This causes the sample to reach the detector somewhat diluted, which results in a broader peak. The total dispersion rate is always an enumeration of different factors, as all disturbances in flow path can cause dispersion, such as valves and fittings.

The dispersion rate can vary between the different injection principles, as the way the sample is stored in the sample loop affects the rate of dispersion that occurs in an autosampler. Figure 6 shows an example of dispersion in a sample loop. In this figure, the sample (orange) is in contact with the mobile phase (blue), causing the sample plug to dilute at both ends.

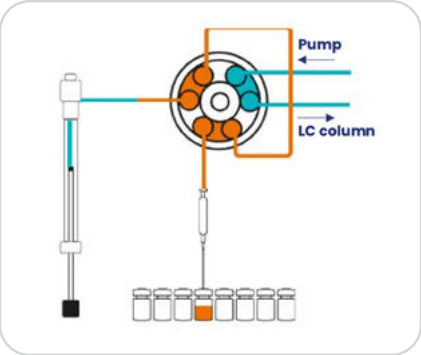
Dispersion in practice

Dispersion is commonly expressed as a system specification, as it is not only autosampler (injection principle) dependent. Dispersion will occur at each component of the LC system that the sample flows through, which means that the column, tubing, and detector play a part as well. Besides this, autosampler dispersion is generally eliminated by the stationary phase in the LC-column and the gradient of the mobile phase. When a compound reaches the column, it will stick to the stationary phase as long as the affinity for this phase is higher than the affinity for the mobile phase. This means that, when using a proper column and gradient for the component, the effects of autosampler dispersion on the sample plug will be minimized.

On the other side, components that do not have a high affinity for the stationary phase (early eluters) may experience some influence due to dispersion caused by the autosampler.

2 Injection principles in autosamplers

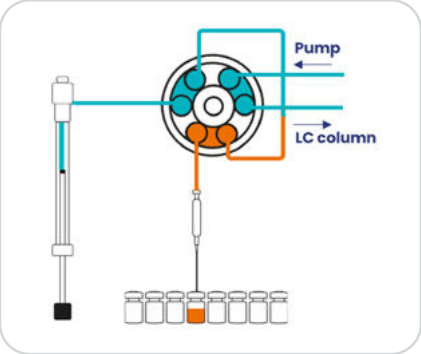
This chapter elaborates on the theoretical differences between all injection principles. Here, a more elaborate representation of the steps needed in order to perform an injection for all injection principles mentioned in this section is shown.



Fixed Loop – Full Loop

Full Loop injections provide the most precise injections, as the injection volume is only dependent on the loop volume; the entire loop content is injected onto the column. The Full Loop injection principle does therefore also not have a contribution to peak dispersion, because the sample plug is 'cut off' at both ends.

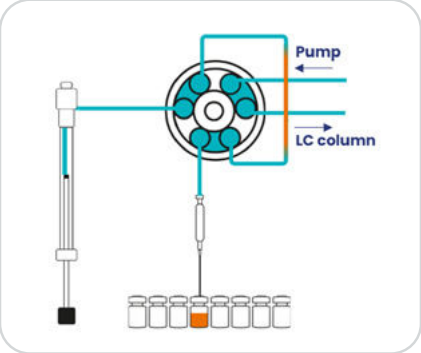
Full Loop does however have downsides. This injection principle does not have much flexibility in injection volume. In addition, the overfilling of the loop results in a lot of sample waste.



Fixed Loop - Partial Loopfill

With Partial Loopfill injections, a part of the loop can be filled, using a syringe or metering device. The partial loop mode offers more flexibility in injection volume compared to a full loop setting. This is because it doesn't require a physical change of sample loop. This injection principle also has a lot less sample loss, as no overfill is needed.

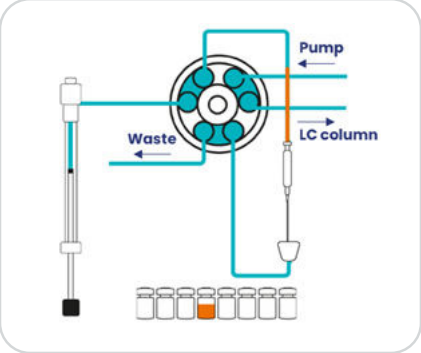
The analytical performance of the Partial Loopfill injection principle relies on the accuracy and precision of the syringe or the metering device, as it directly affects the reproducibility.



Fixed Loop - Microliter Pickup

With Microliter Pickup, the entire flow path is filled with a transport solvent, encapsulating the sample plug. This transport solvent is compatible with the mobile phase used for eluting. With this injection principle, only the amount of sample used for measurement is aspirated, meaning it results in zero sample loss.

The Microliter Pickup injection principle is a more complex injection principle compared to the Partial Loopfill, as it involves more steps to aspirate the sample. This has an effect on the analytical performance, as this injection principle is for example prone to dispersion because the sample plug is exposed to the mobile phase at both ends.



Split Loop

With Split Loop injections, the needle forms a part of the sample loop. With this injection principle, the main advantage is that the sample is directly stored in the loop after aspiration. By design, the Split Loop injection principle does not cause any sample loss. When comparing to Microliter Pickup injections, the Split Loop injection principle has the advantage that the dispersion rate is minimized.

Figure 7: Schematic representation of all injection principles

3. Analytical performance

Reproducibility

Experimental setup	
Flowrate:	0.5 mL/min
Mobile phase:	20% MeOH, 80% water
Injection volume:	10 µL and 1 µL
UV-wavelength:	254 nm
Wash solvent:	20% MeOH, 80% water
Sample:	100 µg/mL cafeïne in 5% MeOH
Column:	C18 2.1 x 50 mm

Table 1. Experimental setup reproducibility experiments

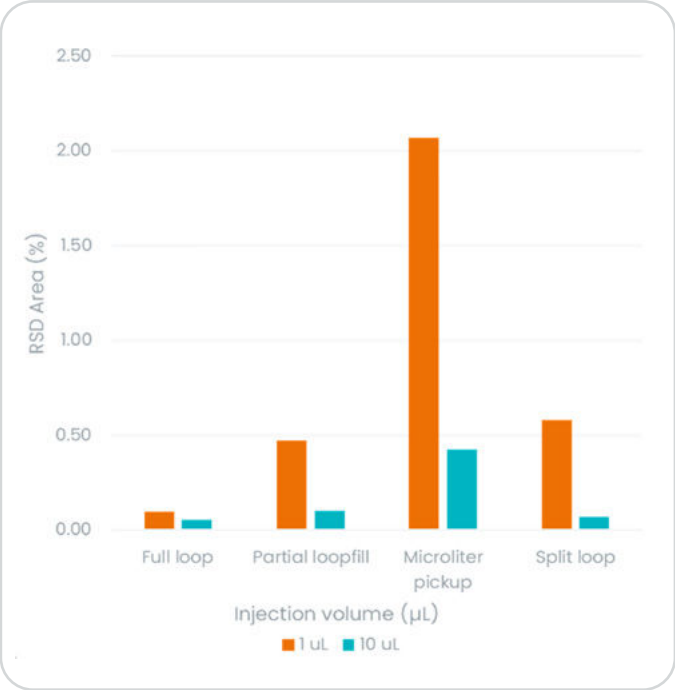


Figure 8: Reproducibility of 10 µL and 1 µL injections

To determine the autosampler reproducibility, a 100-ppm caffeine sample is injected 10 times and the relative standard deviation (RSD) over the resulting peak areas is calculated. This is done for both 10 µL injections as well as 1 µL injections. These experiments have been conducted using all injection principles.

Figure 8 shows the results of these experiments. Full Loop yields the most reproducible results. This is because of the fact that the entire loop content is injected onto the column, which means that the injected volume will always be the same.

These results also show that when injecting the same sample in smaller volumes, the RSD increases. This is because of the fact that with small volume injections, aspiration requires great precision, as discrepancies with smaller values result in greater variances.

These results also show that the microliter pickup injection principle shows a higher RSD compared to the other injection principles. This can be explained by the fact that this injection principle, as mentioned earlier in this paper, can be more prone to peak dispersion. This can lead to more discrepancies in peak integration, resulting in a higher variance of peak areas.

Linearity

Experimental setup	
Flowrate:	0.5 mL/min
Mobile phase:	20% MeOH, 80% water
Injection volume:	10 µL and 1 µL
UV-wavelength:	254 nm
Wash solvent:	20% MeOH, 80% water
Sample:	100 µg/mL cafeïne in 5% MeOH
Column:	C18 2.1 x 50 mm

Table 2. Experimental setup linearity experiments

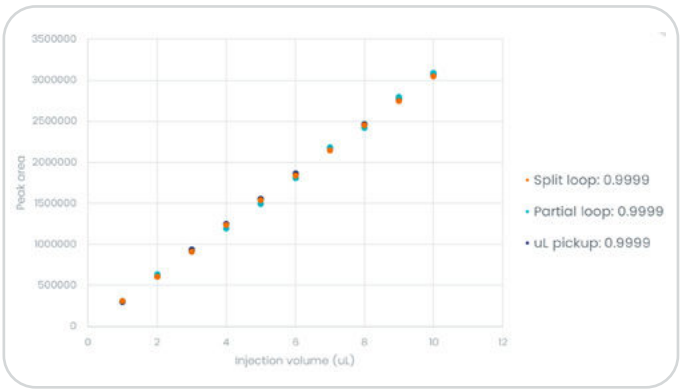


Figure 9. Overlay linearity plot all injection principles

The linearity of the autosampler is measured using a 100-ppm caffeine sample. This sample is injected 10 times with 1 µL increments of injection volume (1-10 µL). To express linearity, the correlation coefficient (r^2) is calculated.

As figure 9 shows, all injection principles show the same linear correlation.

Linearity plot offset

While the r^2-value can indicate a high linear correlation, it does not directly mean that the syringe or metering device is accurate. The measurement of autosampler linearity can not only be beneficial to get an idea of the linearity of the injection volumes but can also give an indication concerning a possible bias in sample aspiration. The offset of the line can be calculated, which could show that the syringe or metering device has a bias, affecting the injection volume accuracy.

Dispersion

Experimental setup	
Flowrate:	0.5 mL/min
Mobile phase:	70% ACN, 30% water
Injection volume:	1 µL
UV-wavelength:	273 nm
Wash solvent:	90% ACN, 10% water
Sample:	150 µg/mL cafeïne in 90% ACN
Column:	No column, 50 µm ID PEEKsil tubing

Table 3. Experimental setup dispersion experiments

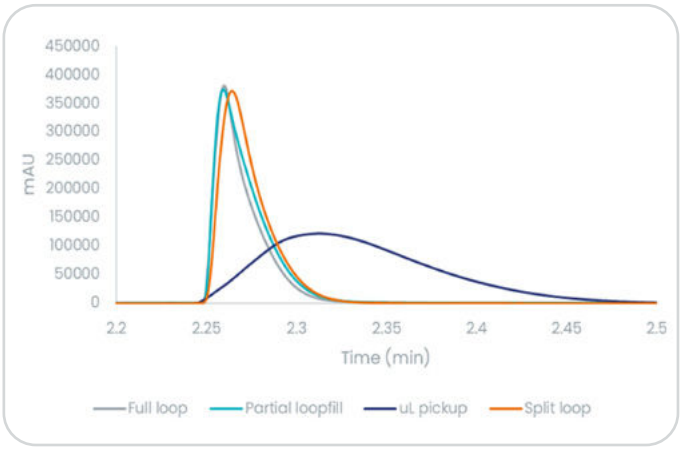


Figure 10. Dispersion chromatogram

The autosampler dispersion is measured using a 150-ppm caffeine sample, with an isocratic elution on a low flowrate. The autosampler is directly connected to a UV-detector without an LC-column. To minimize secondary effects contributing to the dispersion rate, a piece of tubing with a small internal diameter is used. These experiments have been performed for all injection principles, under the same circumstances. Figure 10 shows an overlay of the chromatograms yielded using the three different injection principles.

This figure clearly shows that although the peak areas of all peaks are the same, the Microliter Pickup injection principle yields a different peak shape compared to the Full Loop, Partial Loopfill and Split Loop principles. The shape of the peaks can be explained by the fact that that both Partial Loopfill and Split Loop have the sample plug cut off at one end, (see figure 7) the peaks show no fronting. For the Microliter Pickup injection principle, the sample is stored in the middle of the sample loop, which results in the peak being much broader at both sides.

The Full Loop injection principle was expected to be least affected by peak dispersion, because of the fact that with this injection principle, the sample plug is cut off at both ends, yielding a very sharp and gaussian peak shape. This does however not appear to be the case, as the peak shape using a full loop injection principle is the same as with the Partial Loopfill and Split Loop injections. These results indicate that dispersion mainly occurs when sample is flushed out of the loop, resulting in dispersion at the end of the sample plug.

Experimental setup	
Flowrate:	0.5 mL/min
Mobile phase:	Gradient: 30% ACN – 90% ACN, 70% water – 10% water
Injection volume:	1 µL
UV-wavelength:	254 nm
Wash solvent:	35% ACN, 65% water
Sample:	50 µg/mL acetophenone, butyrophenone, valerophenone in 20% MeOH
Column:	C18 2.1 x 50 mm

Table 4. Experimental setup dispersion in practice

Dispersion in practice

As discussed earlier in this paper, the dispersion caused by the autosampler can be minimized by the focusing effect on the sample plug due to the influence of the LC column and the mobile phase gradient. To visualize this, experiments have been conducted using a 50-ppm sample of acetophenone, butyrophenone and valerophenone. These components all have increasing levels of affinity for the stationary phase. This experiment compares Microliter Pickup and Split Loop injection principles.

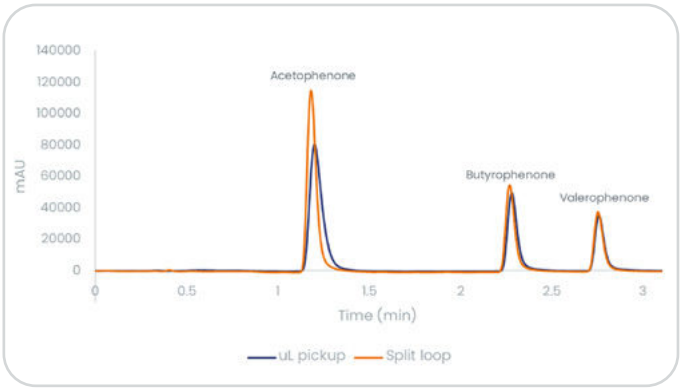


Figure 11. Phenone mix chromatogram

As shown in figure 11, butyrophenone and valerophenone show minimal difference in peak shape. Influence of autosampler dispersion on peak shape is most noticeable on components that have a lower affinity for the stationary phase. Acetophenone shows a difference in peak shape between Split Loop and Microliter Pickup, resulting, in this situation, in a 30% sensitivity loss when using Microliter Pickup. These results do show that autosampler dispersion rate is entirely application dependent. This means that when measuring early eluting components, Split Loop or Partial Loopfill might be the better option compared to Microliter Pickup.

4 Conclusions and recommendations

The results discussed in this paper provide insight into the comparison of different (U)HPLC injection principles, based on analytical performance.

Reproducibility

While full loop is still theoretically the most reproducible injection principle, the Partial Loopfill and the Split Loop injection principles prove to yield comparable results, with Split Loop having the advantage of resulting in no sample loss. For the Fixed Loop injection principles, avoiding sample loss is only possible using Microliter Pickup injections. This injection principle yields slightly less reproducible results but is a good alternative when working with limited sample amounts.

Linearity

Fixed and Split Loop injection principles show comparable results concerning linearity, with all injection principles performing yielding impressively high r^2 values.

Dispersion

When comparing all injection principles, Split Loop, Partial Loopfill and Full Loop show to yield comparable results concerning dispersion rate. When measuring compounds that have an affinity for the stationary phase used for separation, the effects of autosampler dispersion are reduced. When measuring very early eluting compounds however, Microliter Pickup injections has the most influence on the dispersion rate, which could lead to loss in sensitivity.

This white paper shows that the best injection principle is strongly dependable on the application. The table below provides an overview of the results and gives a recommendation of which injection principle to use.

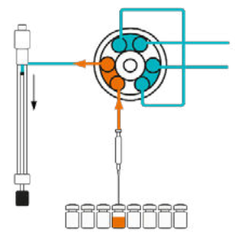
Injection design	Reproducibility	Linearity	Dispertion	Sample loss	Application
Fixed Loop Full Loop	Very high	N.A.	Low	High	• High precision • Fixed injection volume
Fixed Loop Partial Loopfill	High	Very high	Low	Medium	• Precise runs • Low dispersion rate
Fixed Loop Microliter Pickup	Medium	Very high	High	None	• Small injection volumes • No sample loss
Split Loop	High	Very high	Low	None	• Precise small injection volumes • Low dispersion rate • No sample loss

Table 5. The comparison of different injection routines in (U)HPLC autosamplers

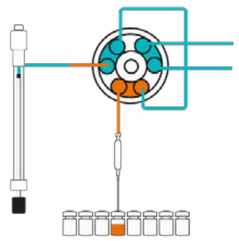
Injection principles in (U)HPLC

Full Loop

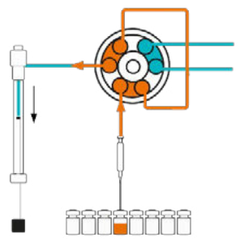
Step 1
- Aspirate preflush
- Preflush flows to waste after injection



Step 2
- Switch valve to LOAD



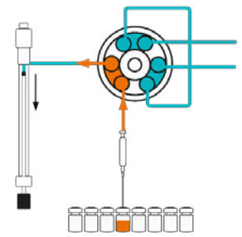
Step 3
- Aspirate sample by overfilling sample loop



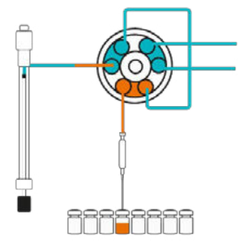
Inject sample

Partial Loopfill

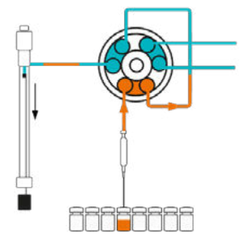
Step 1
- Aspirate preflush
- Preflush flows to waste after injection



Step 2
- Switch valve to LOAD



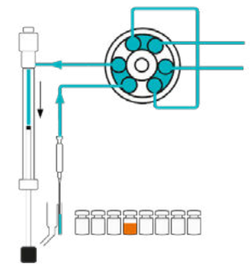
Step 3
- Aspirate sample



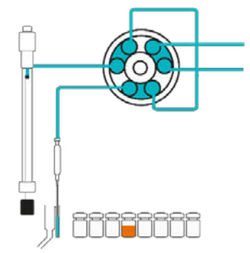
Inject sample

Microliter Pickup

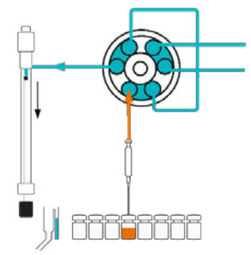
Step 1
- Aspirate 1st transport solvent



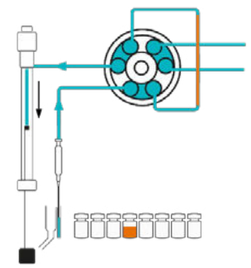
Step 2
- Switch valve to LOAD



Step 3
- Aspirate sample



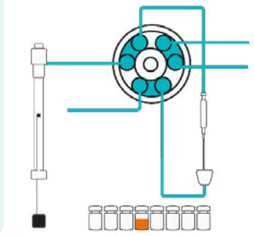
Step 4
- Aspirate 2nd transport solvent



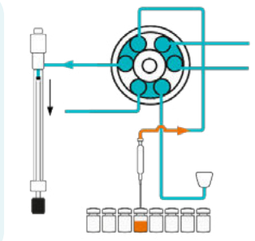
Inject sample

Split Loop

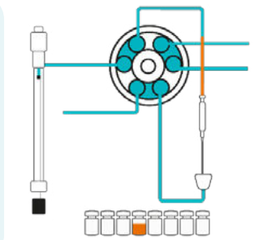
Step 1
- Switch valve to LOAD



Step 2
- Aspirate sample



Step 3
- Move needle to needle seat



Inject sample