



WHITE PAPER JULY 2022

## **Comparison of high-end injection valves for UHPLC autosamplers:**

A benchmark for quality, performance and lifetime

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# Table of contents

## 1. Overview

## 2. Injection valves in autosamplers

- A. Construction & operation
- B. Pressure range
- C. Materials & coatings
- D. Injection valve performance

## 3. Benchmark of injection valves

- A. Methodology
- B. Experimental conditions

## 4. Performance (results)

- A. Initial & long-term performance
  - repeatability/reproducibility
  - carryover
- B. Wear
  - Spark
  - Vendor A
  - Vendor B

## 5. Comparison & conclusion

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# 1. Overview

The injection valve is one of the most important parts of the autosampler. The choice of an injection valve for an autosampler is primarily determined by performance and lifetime. Companies that supply autosamplers to laboratories usually equip them with a self-produced injection valve or a valve from a reputable brand.

Several UHPLC valve manufacturers are considered as high-end suppliers. Spark is an OEM provider of UHPLC valves and therefore often compared with the well-known vendors.

Is there an alternative for UHPLC valves of high-end suppliers that performs at least as well and lasts as long? Until now no systematic research has been conducted to satisfactorily answer that question.

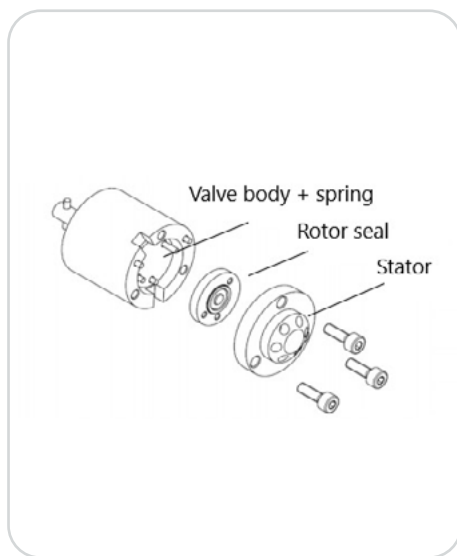
To answer this question, a benchmark in collaboration with NHL Stenden University has been conducted comparing high-end injection valves from different brands. This study is conducted to demonstrate the quality, performance and lifetime of Spark UHPLC valves compared to other well known, high-end UHPLC valve manufacturers. On the basis of the benchmark the conclusion is justified that the Spark UHPLC injection valve is an equally good, if not better, alternative for other available high-end valves.

This white paper provides insight into the approach and results of the benchmark. It helps to make a more considered choice of injection valves for your UHPLC autosampler in order to optimally advise and support laboratories.

## 2. Injection valves in autosamplers

The injection valve is the heart of the autosampler in an UHPLC system. In this publication, the valves with 6 ports and 2 positions will be discussed, because these valves are the most common to inject a sample.

### A. Construction & operation



An injection valve consists of a valve body with a stator and a rotor seal, see Figure 1. To get a leak-free connection between the rotor and stator, the rotor is pushed against the stator by the spring in the valve body.

The front of the valve, where the tubing can be connected, is the stator. The stator consists of 6 ports, 2 ports in the stator are connected through the grooves in the rotor seal. The rotor has 3 arched flow passages (grooves) to connect the stator ports. The rotor seal rotates in the valve when the valve switches from position (INJECT / LOAD). The default position is the INJECT position. To introduce the sample into the flow path, the valve switches from INJECT to LOAD, see Figure 2. In the LOAD position, the syringe aspirates the sample into the loop. After the sample is loaded into the loop, the valve switches back to INJECT. The sample in the loop will be transferred by the eluent into the column.

Figure 1, Injection valve schematic

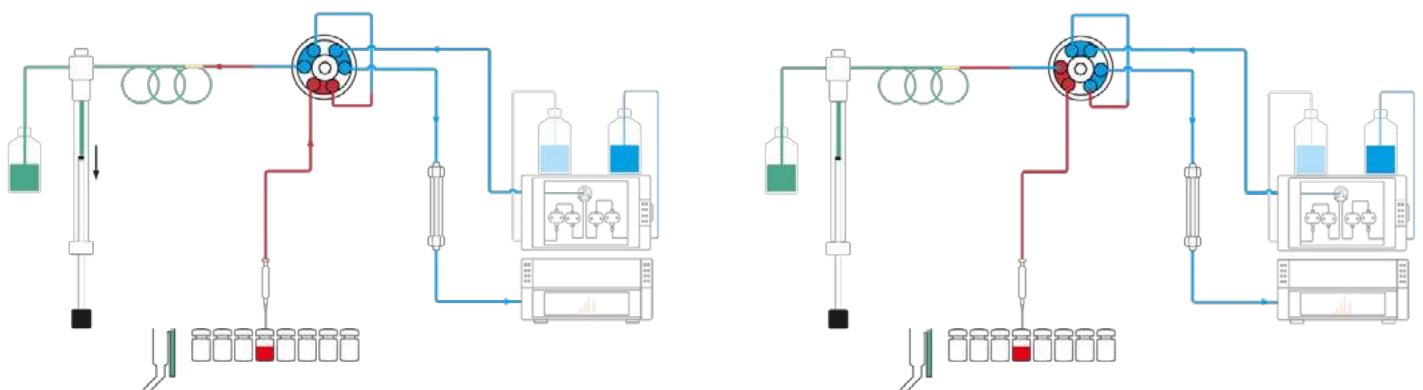


Figure 2, Positions and flow direction of an injection valve

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## 2. Injection valves in autosamplers

### B. Pressure range

For more than four decades, reducing stationary phase particle size has been investigated to improve chromatographic separation efficiency. Until the early 2000's, Liquid Chromatography (LC) technology had reached a plateau, in which the benefits of reduced particle size could not be realized due to limited pressure range of the HPLC system. In 2004, separation science was revolutionized when Waters introduced the first Ultra-Performance Liquid Chromatography system. Advances in instrumentation and column technology were made to achieve dramatic increases in resolution, speed and sensitivity. Not long after, vendors (e.g. Spark Holland) entered the LC market with similar systems which could handle pressures up to 18.000 psi.

As the injection valve is part of the LC flow path, this led to new designs of the injection valves. Not only the maximum pressure range was increased, but also internal volumes were decreased. The internal volumes are kept as small as possible, so this will not affect the increased separation performance due to the smaller column particles.

### C. Materials & coatings

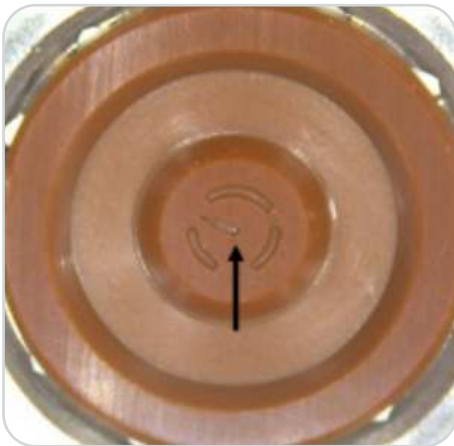
The rotor seal determines the performance of the valve (carryover takes place within the rotor seal and can occur due to a damaged stator). The rotor seal rotates continuously and has to be replaced after a certain number of rotations (part of yearly maintenance). Rotor seal wear can cause the performance to deteriorate. Rotor seals predominantly consist of softer materials than stators to get a leak-free connection, like Polyimide, PTFE or PEEK. Stators usually consist of materials such as stainless steel (SS), PEEK or titanium. To receive a robust and tight connection the stator is often coated. The coating also increases the lifetime of the valve. Diamond-like carbon (DLC) coatings have emerged as the ideal solution for demanding tribological applications where components are under high loads or subject to extreme friction, wear and contact with other parts. In these types of environments, only the high hardness of a DLC coating – along with a corresponding low coefficient of friction – can prevent parts from pitting, galling, seizing and ultimately failing in the field. DLC coating reduces friction and increases the lifetime of a stator.



## 2. Injection valves in autosamplers

### D. Pressure range

In UHPLC chromatography it is important to maintain a constant pressure to increase the lifetime of the column, and to avoid sample dilution at the moment the valve is switched. To optimize the performance, valve manufacturers have optimized the design of the injection valve the recent years. Currently, some improvements that may be found in an injection valve are Make-Before-Break (MBB) and Pre-pressurize loop. Another innovation, which is particularly noteworthy, is Intermediate Loop Decompression, patented by Spark Holland (ILD™).



**Figure 3, ILD injection valve rotor seal, arrow pointing to the additional radial groove in the center of the rotor seal**

At the start of analysis, prior to sample aspiration into the sample loop, the injection valve is in the inject position. In this position, the sample loop is under ultra-high pressure as mobile phase is flowing from the LC pump through the sample loop to the LC column. For full loop and partial loop fill injection modes, when the injection cycle starts, the syringe fills the needle and some of the buffer tubing with sample to prepare to load the sample loop. The injection valve switches to the load position causing the compressed liquid in the sample loop to expand at the outlet, into the sample needle. This results in an uncontrolled dilution of the sample at the inlet of the sample loop, and although the syringe draws a correct sample volume into the loop, part of the sample has been diluted owing to the change from high pressure to ambient pressure as the valve switches. Introducing a sample volume into the UHPLC system that has been partially diluted results in peaks that represent an inaccurate analyte concentration.

The ILD injection valve, patented by Spark Holland, is a 7-port valve with a strategically placed radial groove in the rotor seal (Figure 3). This radial groove is connected to an additional waste outlet port in the center of the valve. This allows for decompression of the sample loop in milliseconds when the valve switches position from ultrahigh pressure (inject) to ambient pressure (load). When the injection valve switches into the load position, the radial groove passes by one of the sample loop ports (position 2 on the valve). The liquid in the sample loop is then allowed to expand via the central outlet port to waste, releasing pressure. Therefore, the sample loop will be at ambient pressure as the valve continues to switch and arrives at the load position.

Thus, the ILD injection valve ensures that an accurate sample volume will be injected onto the UHPLC system every time. The result is excellent reproducibility and longer column lifetime.



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## 3. Benchmark of injection valves

This benchmark is a long term analytical performance comparison of the Spark injection valve with two other high-end UHPLC valves (vendor A, and vendor B). All three valves are well known OEM injection valves in the UHPLC market, and have a good reputation. All three valves have a diamond-like carbon (DLC) coating or some variant thereof but use a different rotor seal material.

### A. Methodology

All three valves have been installed in a common UHPLC system as the only part to be exchanged for these experiments. Each valve is used up to 30,000 injections under UHPLC conditions at 75 – 85% of the maximum pressure of the valve. After each 5,000 injections, the valve is inspected for analytical performance and after each 30,000 injections a visual inspection of wear was carried out. Every injection valve has been tested using three rotor seals to test the lifetime of the stator. This makes a total of 90,000 switching cycles per injection valve. The performance of the injection valves is determined by measuring injection repeatability of the peak area and carryover of caffeine. In doing so a maximum reproducibility ( $< 0.3$ ) and minimal carryover ( $\leq 0.005\%$ ) was expected.

#### 1. Injection repeatability

The repeatability (or reproducibility) is the precision of an instrument or method under the same circumstances. When the injections of a valve are not repeatable, for example caused by intensive wear, the results are not precise. To determine the repeatability of the injection valves one sample was injected 10 times from the same vial. The repeatability is expressed in the relative standard deviation, RSD.

#### 2. Carryover

Carryover is a type of sample contamination in the UHPLC system. When injecting a sample a small part may remain behind. When injecting the next sample, the sample will take this small remaining to the column. Resulting in a sample peak of a component that is not present in the sample. Besides wear can aggravate carryover. The carryover is expressed in a percentage of the concentration of the previous injection. In this benchmark carryover of caffeine was investigated.

### 3. Benchmark of injection valves

#### B. Experimental conditions



Figure 4, Schematic drawing of the analytical system

The repeatability and carryover were determined using a stepwise protocol, using the Spark UHPLC system:

1. Switch on the pump and program a flow rate of 0.5 ml/min.
2. Auto zero the UV-detector.
3. Start the sequence as indicated in Table 1.
4. Calculate the RSD of the peak areas of injection 5 to 14.
5. Calculate the carryover of injections 16, 17, and 18.
6. Check if the RSD and carryover meet the following targets:
  - a. RSD  $\leq$  0.3%.
  - b. Carryover  $\leq$  0.005%.

A schematic overview of the system is shown in Figure 4.

In Table 1 the experimental setup is listed.

Solvents	
<b>Wash solvent autosampler:</b>	20/80 methanol/water
<b>Eluent:</b>	20/80 methanol/water
<b>Flow</b>	0.5 mL/min
<b>Sample - blank:</b>	Water
- low:	0.5 ppm caffeine in water
- medium:	50 ppm caffeine in water
- high:	10,000 ppm caffeine in water
Method	
<b>Injection:</b>	Full loop fill
<b>Preflush volume:</b>	30.0 $\mu$ L
<b>Air segment:</b>	Off
<b>Analysis time:</b>	2 minutes (blank, low, medium), 6 minutes (high)
<b>Wash volume inside:</b>	250 $\mu$ L
<b>Wash volume outside:</b>	30.0 $\mu$ L

Table 1, Experimental setup (solvents & methods)

The sequence for measuring the repeatability and carryover is shown in Table 2.

Injection	Sample	Injection	Sample
1	Blank	7	Medium
2	Blank	8	Medium
3	Low	9	Medium
4	Low	10	Medium
5	Medium	11	Medium
6	Medium		

Table 2, Sequence for repeatability and carryover

## 4. Performance (results)

### A. Initial & long-term performance

To make a comparison of the initial and long-term performance two indicators have been applied: repeatability and carryover.

#### Repeatability/reproducibility

As a target, a repeatability of  $\leq 0.30\%$  was set. This target is based on the

specifications of the integrity auto samplers with the Spark injection valves. Figure 5 shows that the injection valves in general meet the target and perform equally. The repeatability of the injection valves is mainly around 0.1%. The repeatability of the injection

valves does not decrease for 30,000 injections. This is for the new injection valve, the second rotor seal and the third rotor seal almost identical. This indicates that the repeatability of the injection valves does not decrease due to wear on the stator within 90,000 injections.

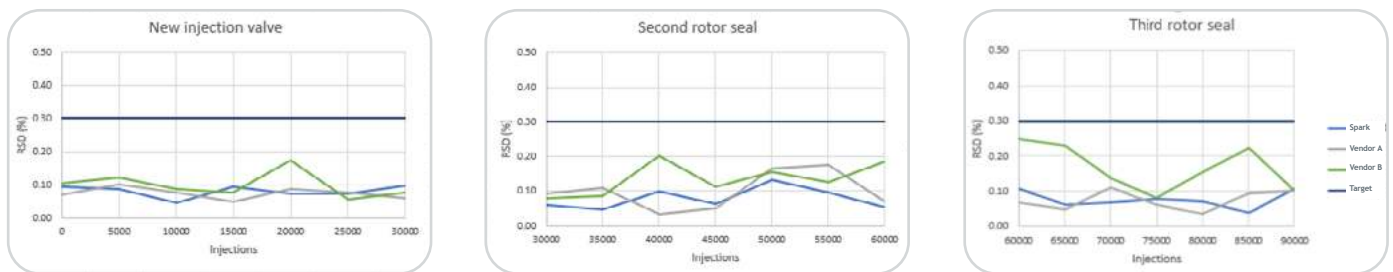


Figure 5, All injection valves meet the repeatability target of  $\leq 0.30\%$

#### Carryover

As a target, a carryover of  $\leq 0.005\%$  is set. This target is based on the specifications of the integrity autosampler with the Spark injection valves. Figure 6 shows that the

injection valves in general meet the target. The carryover of the injection valves does not increase for 30,000 injections. This is for the new injection valves, the second rotor

seal, and the third rotor seal almost identical. This indicates that the carryover of the injection valves does not increase due to wear on the stator within 90,000 injections.

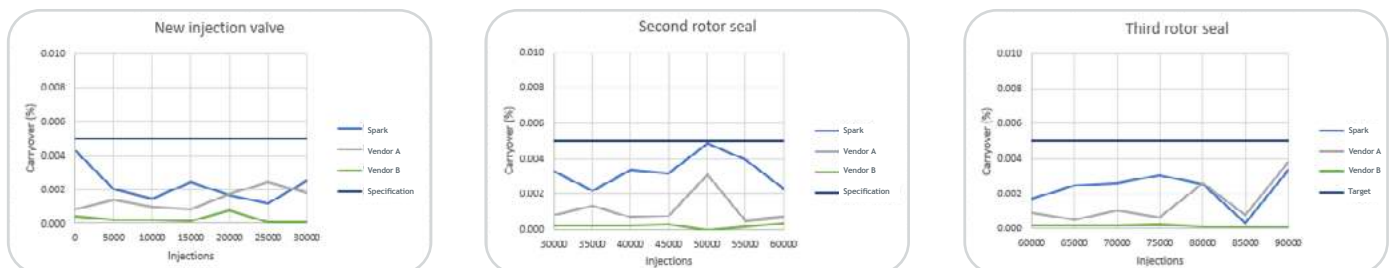


Figure 6, All injection valves meet the carryover target of  $\leq 0.005\%$

## 4. Performance (results)

### B. Wear

Wear of the rotor seal or stator may result in leaking and may impact the analytical performance of the injection valve. After every 30,000 injections, the rotor seal and stator were examined using a microscope.

#### Spark

##### Rotor seal

The used rotor seals of the Spark

injection valve all show some discolorations and superficial wear after 30,000 injections, see figure 7.

These light traces however have no consequences for the analytical performance of the injection valve.

##### Stator

After disconnecting the stator of the rotor seal and the body of the injection valve the stator seemed

affected by the injections. It turned out to be rotor seal material on the stator. After cleaning the stator with tissue and acetone there were no traces left of the rotor seal material. From microscope pictures and the analytical performance may be concluded that the Spark stator can be used for 90,000 injections without any problems, under comparable circumstances.

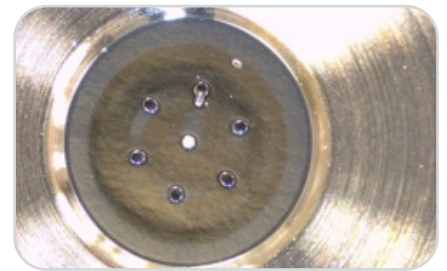


Figure 7, The Spark rotor seal shows some superficial wear after 30,000 injections. The stator shows no wear after 90,000 injections. There are no consequences for the analytical performance



## 4. Performance (results)

### Vendor A

#### Rotor seal

The used rotor seals of the Vendor A injection valve show some discolorations and deformations. The rotor seal for 0 to 30,000 injections shows less wear than the rotor seal for 60,000 to 90,000 injections. This might conclude that the state of the stator and/or valve body decreased, causing the rotor seal to wear faster. The used Vendor A rotor seals have a circle of wear around the grooves, while the new rotor seals were clean. Another observation is the wear

between groove 2 and groove 3. The Vendor A injection valve met the target for repeatability and carryover up to 90,000 injections. This concludes that the wear between groove 2 and groove 3 for the Vendor A rotor seals is superficial and does not impact the analytical performance of the injection valve.

#### Stator

On microscope pictures no wear is

visible. There are some scratches visible in the middle of the stator, but these scratches do not seem to have consequences since the Vendor A injection valve met the target for the analytical performance. From the microscope pictures and the analytical performance of the Vendor A may be concluded that the Vendor A stator can be used for 90,000 injections without any problems, under comparable circumstances.

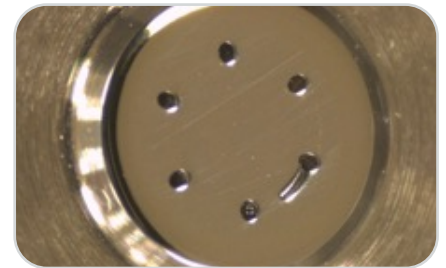


Figure 8, The Vendor A rotor seal and stator show some traces, but they have no consequences for the analytical performance



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## 4. Performance (results)

### Vendor B

#### Rotor seal

The used rotor seals of the Vendor B injection valve show some discolorations. The discolorations of the rotor seals are consistent. The Vendor B valve show wear between groove 1 and groove 2. Since the eluent did not leak out of the sample needle and the injection valve did meet the targets for the analytical performance for the Vendor B up to 30,000 injections, it can be concluded that this wear is superficial and does not impact the analytical performance

of the injection valve. Also some wear at the bottom of groove 2 was observed. It may be concluded that this wear is superficial and does not impact the analytical performance of the injection valve.

#### Stator

The Vendor B hardly had some rotor seal material on the stator. The Vendor B stator was cleaned with a tissue and isopropyl alcohol. Microscope pictures show some

wear on the stator of the Vendor B injection valve. This wear seems to increase after more injections are made. The wear is visible as bright spots in the microscope pictures. These bright spots are most likely created because the coating has crumbled off the stator. After 30,000 injections the first spots are shown. But the spots stand out after 50,000 injections. Except from these bright spots the stator does not show any other signs of wear.



**Figure 9, The Vendor B rotor seal shows some light wear on the low pressure side and the stator shows a damaged coating, but they have no consequences for the analytical performance**



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## 5. Comparison & conclusion

A systematic and thorough research has been conducted comparing high-end injection valves from different brands (Spark, Vendor A, and Vendor B). The benchmark was conducted transparently and the results are reliable and verifiable.

### Repeatability

The Spark, Vendor A, and Vendor B injection valves meet the target for the repeatability. There is no trend visible for a decreasing repeatability at an increasing amount of injections. The repeatability of the Spark, Vendor A, and Vendor B are comparable, under these circumstances.

### Carryover

The Spark, Vendor A, and Vendor B injection valves in general meet the target for the carryover. The carryover remains stable from 0 to 90,000 injections. The Vendor B injection valves seem to have less carryover than the Vendor A, and Spark injection valves. The carryover of the Vendor A, and Spark injection valves is between 0.001% and 0.005%, while the carryover of the Vendor B injection valves is below 0.001%. This difference in carryover might be caused by the lack of the Make-Before-Break function of the Vendor B injection valve.

### Wear

The rotor seals for the Spark, Vendor A, and Vendor B injection valves all show signs of use. The Vendor B rotor seals seems to have less wear (and discolorisations) than the Spark, and Vendor A rotor seals. The Spark, Vendor A, and Vendor B rotor

seals show the most wear between the grooves on the ambient pressure side of the injection valve. The stators of the Spark, and Vendor A injection valves show hardly any wear. The Vendor B injection valve however does show wear in the form of crumbled off coating close to three ports. This damage has resulted in a leaking injection valve.

### Conclusion

On the basis of the benchmark, the following general conclusions are justified:

- Spark injection valves last their performance without any decrease up to 90,000 injections, after replacing the rotor seal every 30,000 injections.
- Spark rotor seals show only some discolorations and superficial wear, but this does not impact the analytical performance of the injection valve.
- Spark stators do hardly show any wear after 90,000 injections, and do not impact the analytical performance of the inspection valve.
- Spark injection valves, compared with the Vendor A and Vendor B valves, do not show substantial problems, like leakage causing wear and subsequent decrease in analytical performance.

**The Spark UHPLC inspection valve is an equally good, if not better, alternative for other available high-end valves. There is no objective reason why companies supplying autosamplers to laboratories would limit themselves to autosamplers with an injection valve from a reputable brand.**

