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VANQUISH NEO

TIPS AND TRICKS: ISSUE 1

How to avoid and recover from autosampler blockages

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Keywords

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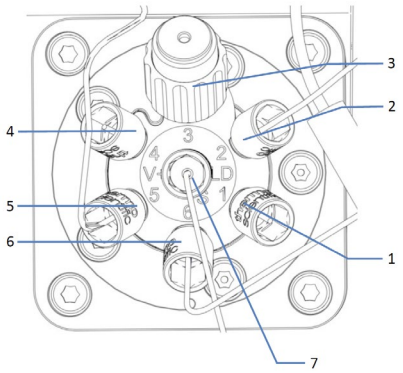
Goal

Better understanding of the purpose of the autosampler needle seat and the procedures to remove potential blockages

Introduction

The Thermo Scientific™ Vanquish™ Neo UHPLC system supports applications from micro flow (≤100 µL/min) down to low-nano flow (≥100 nL/min). For low flow LC based applications (and nanoLC in particular), minimizing the gradient delay volume (GDV) is essential for obtaining fast and efficient separations. For this reason, all system fluidic connections are designed to be as short as possible, making use of narrow inner diameter capillaries. Because of this, the fluidic path is more susceptible to blockages from particulates and other insoluble components. The needle seat of the Vanquish Neo autosampler represents the 1500 bar pressure capable interface between the sampler needle unit and the autosampler switching valve. The needle seat contains a 0.5 µm filter frit, which prevents insoluble components, which may have been drawn up into the needle from entering the system. As the first line of system defense against particulates, it protects all downstream components (e.g. switching valves, capillaries, and columns) from blockage and / or damage. For this reason, the needle seat requires regular cleaning and / or replacement, the frequency of which will depend on the sample matrix, the consumables used as well as the sample throughput.



1: Viper plug
2: Solvent valve
3: **Needle seat**
4: Column (if installed: Column Compartment)
5: Sample loop
6: Metering device
7: Pump

Figure 1. Injection valve of the Vanquish Neo autosampler with connected capillaries in the Direct Injection workflow and needle seat on port 3.

Here we describe best practice guidelines to reduce the frequency at which needle seat blockages occur. Furthermore, we detail the automated and semi-automated Vanquish System Controller based maintenance, diagnostic and troubleshooting scripts which can be used to diagnose blocked needle seats as well as how to backflush them.

Guideline

1. How to avoid introducing particles into the system

Insoluble components, which can block the needle seat frit, are mainly introduced into the system during the injection process from either the sample or components involved during the injection.

- Particles from vials, vial septa, well plates and covers

Some sample vial cap septa products include solid filler components (e.g., talcum and seal-filler particles) which can be shed into the vial during the septa piercing process and subsequently introduced into the system during sample pickup. For this reason please only use talcum-free, unfilled silicone/PTFE septa (septa PN: 6PSC9STB1 or 100 vials + septa PN: 6PK1655).

Well plate cover mats should be unfilled silicone-based. If well plate sealing tape is used this should be without adhesive at the cavity area (PN: 60180-M176). Also sealing mats that are likely to result in seal material being punched out thus ending up inside the autosampler needle must be avoided.

Please note that septa generally only support a limited number of repeat injections, typically <5 per septum. Increasing the number of repeat injections per septum greatly increases the risk of introducing septa related particles into the sample vial and consequently into the needle seat.

- Particles from sample preparation steps

The usage of reversed phase C18 cartridges for sample purification can lead to the release of stationary phase material into the final sample. Injecting samples contaminated with stationary phase may lead to needle seat blockages and can also impact the chromatography, as the stationary phase material accumulating in the needle seat frit can also bind sample analytes from subsequent injections. These analytes will subsequently be lost because the needle seat is not in line with the analytical flow path during the gradient phase of the run for the majority of low-flow LC methods.

As such, please use C18 cartridges purification products, which release as little stationary phase material as possible. Alternatively, adapt the sample preparation protocol to include additional centrifugation or filtering steps to avoid stationary phase material is present in the final sample, which is injected into the LC.

The Vanquish Neo autosampler is equipped with a vial bottom detection function, which enables the needle to actively detect the vial bottom and draw the sample from that position. This enables injections of limited sample volumes. It is recommended to keep the vial bottom detection function active, but for samples with insoluble impurities a lower DrawSpeed setting (≤0.2 µl/s) and deactivating the vial bottom detection function can be evaluated (NeedleHeight, default setting: 4 mm). The latter will increase the sample volume which will remain in the vial, but can avoid drawing particles accumulating on the vial bottom. Both values can be modified in the advanced autosampler settings of the Instrument Method Editor.

- Particles from damaged needle unit coating

Mechanically damaged or bent autosampler needle units can lead to damage of the outer needle coating and the release of particles, which can also lead to needle seat or system blockages.

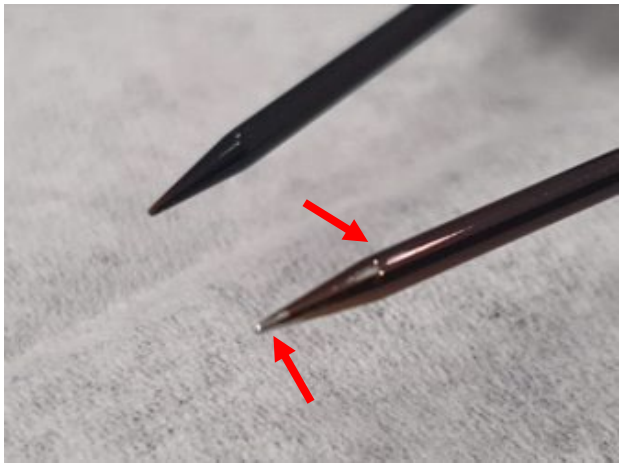


Figure 2 . Comparison between normal needle coating (top) and damaged needle coating (bottom- affected areas are marked with arrows).

Please note – if the needle coating is missing or damaged both the needle seat and the needle unit must be replaced by running script C21 on the Vanquish System Controller and selecting “replace needle unit and seat” under the clean / replace options.

2. Monitoring the system back pressure on a regular basis

The Vanquish Neo system is equipped with a diagnostic script (“D01 - Test System Back Pressure”) which both checks the system backpressure as well as identifying and locating system blockages and/or defective parts causing overpressure. This script should be carried out on a regular base, when changing solvents, whenever the operator encounters an overpressure, but at least every two weeks.

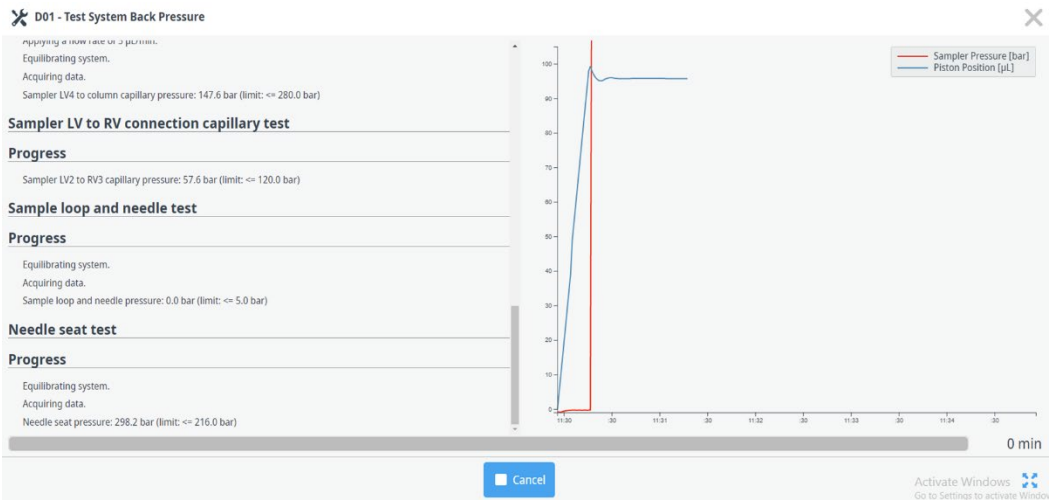


Figure 2. D01 – Test System Back Pressure script with identified blocked needle seat

If the procedure passes the instrument is fully operable, please review and document the results (e.g. via screenshot).

3. Clean needle seat to remove insoluble particles

If an increased backpressure or blockage of the needle seat was identified with the D01 script, please perform script C21 “Clean or Replace Needle Unit and Seat” to remove potential insoluble components. This automatic procedure performs a back flush of the needle seat and then automatically tests the back pressure.

Please note, this script does not guarantee full regeneration of the needle seat functionality, but it may remove some of the particles and bring the needle seat back to an operable backpressure. It may need to be executed multiple times to recover the needle seat from a blockage. The needle seat nevertheless is a consumable. If the cleaning procedures are unsuccessful and the needle seat is permanently and irreversibly blocked, proceed to replace the needle seat following the procedure described below.

4. Replace needle seat

If cleaning and back flushing the needle seat is not successful, or if the needle seat or needle unit is damaged, please use script C21 “Clean or Replace Needle Unit and Seat” to replace the needle seat (PN: 6252.2470) and if required also the needle unit (PN: 6252.1130).

Please note, a damaged needle unit also causes a damaged needle seat. For this reason, it is recommended to replace the needle seat together with the needle unit. However, if the needle seat is blocked but the needle unit is not damaged then it is ok to simply replace the needle seat whilst retaining the needle unit.

For details on how to replace needle seat and needle unit please also refer to the Vanquish Neo System Operating Manual (link, page 242 and page 244).

Conclusion

When operating the Vanquish Neo low-flow UHPLC system, the introduction of particle impurities should be avoided in all stages of the sample preparation and work-up. Please make regular use of the automatic diagnostic and maintenance scripts available on the Vanquish Neo system controller to locate and where possible, remove blockages. If these procedures are not successful, please replace the affected parts using the respective VSC script as required.

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