Ensuring Best Possible UHPLC Performance Through an Innovative Capillary and Fitting Design

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ABSTRACT

Tubing and fitting technology can strongly affect the quality and robustness of HPLC analysis, especially with small column volumes and ultrahigh pressures. At the same time, it is critical that tubing and fittings are easy to work with, as they must be reconnected each time a new column is used in the system. Today, the general challenge is to secure the position of the capillary tip in a tightening ferrule, yet avoid all possible dead volume. When different column hardware is used in the same system, these challenges are multiplied due to varying lengths that the capillary extends out of the ferrule. The recently introduced Viper™ fingertight fitting system shows improved chromatographic results when compared with conventional capillary and fitting technology. Even replumbing to LC hardware of various suppliers and advanced setups with multiple switching valves is easy. The new fitting system does not require special training, but can be used intuitively to connect to any LC column and system hardware.

INTRODUCTION

Extracolumn volumes in HPLC have detrimental effects on the separation performance of an LC system and have to be minimized as much as possible. This is particularly critical with the use of small-volume separation columns with relatively small inner diameters and short lengths. Poor capillary connections can cause peak broadening, peak splitting, and carryover. Therefore, it is crucial for chromatographic performance to establish and maintain optimal connections between all fluidic components in an LC system.

Here, we review conventional tubing and fitting designs with respect to their strengths and weaknesses regarding demands on user skills, safety for long-term zero-dead volume connection at ultrahigh operating pressures, and compatibility with different counterparts. Next, it introduces a new fitting design that addresses many of today's challenges. Instead of using ferrules, this design tightens using a seal at the capillary tip. Simple plumbing for pressures of up to 1,000 bar, zero-dead volume, and compatibility with virtually any column hardware or valve type are guaranteed.

FITTING SYSTEM REQUIREMENTS

Fitting systems are a significant part of each HPLC system and their importance for chromatographic performance is still widely underestimated. Improper use leads to many negative effects and often causes time-consuming troubleshooting. In general, fitting systems must fulfill two major tasks: 1) Seal a connection between two fluidic parts tightly, and 2) lock a capillary in the optimal position with minimized void volume against system pressure in a receiving port (See Figure 1). In summary, the ideal fitting system should be:

- Able to establish a dead-volume free connection of fluidic LC parts, independent of the design of the receiving port
- Robust for long-term use with strict avoidance of capillary or ferrule slippage
- Easy to use through flexible capillaries and fingertight plumbing concept

CURRENT SITUATION

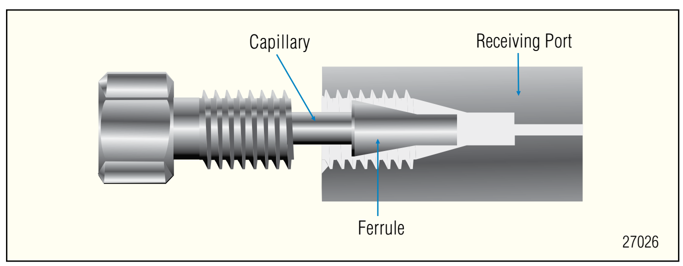

Figure 1. Design of a conventional ferrule-based, two-piece fitting system.

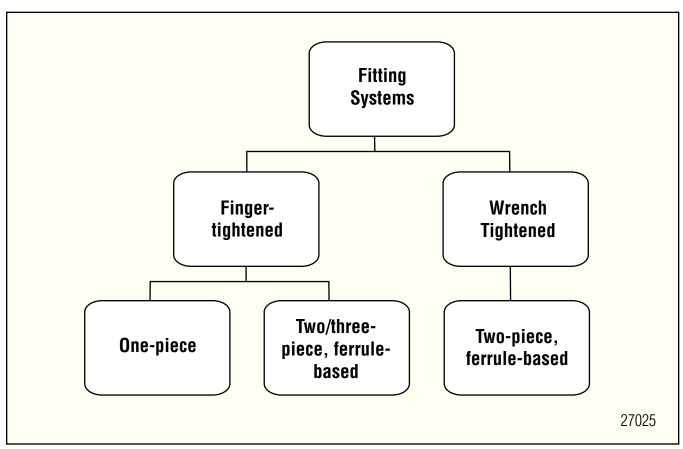

Figure 2. General overview of different high-pressure fitting system families.

A typical standard HPLC system requires at least three external capillaries to connect all modules and the separation column. This results in a minimum of six fitting connections, which can significantly affect the quality of chromatographic separations.

Potential Error Sources During the Use of Fittings

Numerous errors can occur through handling of tubing connections, all of which may compromise the performance of an LC system, including:

- Poor cutting quality of a capillary
- Accidental use of wrong ferrules
- Use of too-small capillary o.d.s relative to the receiving port i.d.
- Incorrect positioning of the ferrule on a capillary, or slipping of the ferrule during use with UHPLC pressures

Since the introduction of UHPLC in 2004, some advanced fingertight fitting systems have been developed and introduced to the market. Some of these designs support the user to properly guide and fix the capillary in the correct position. However, most of them still exhibit certain downsides, including:

- Bulky design
- Requirement of special care and dedicated user training
- Inability to support true UHPLC backpressures (above 600 bar)

The latest solution for fingertight HPLC and UHPLC connections is the Viper fitting system by Dionex, which addresses all current issues of existing HPLC and UHPLC fitting systems available today. It uses a revolutionary ferrule-free tightening concept and guarantees ease of use and zero-dead volume with any capillary connection, independent of the counterpart design. Table 1 compares the properties of conventional fitting systems and the Viper system.

EXPERIMENTAL

Two different HPLC systems were used to perform comparative experiments between the new Viper and conventional capillary/fitting systems. Setup A performed on long-term operation at high backpressure, focusing on extracolumn effects. The performance of Viper versus conventional fittings using advanced column-switching scenarios was examined with setup B. In addition, practical handling tests to assess ease of use and flexibility were performed.

Table 1. Assessment of the Advantages and Disadvantages of Different Fitting System Designs (+): Positive Assessment, (-): Negative Assessment

	Finger-tightened			Wrench-tightened	Consequences of Failure
	One-piece	Two/three-pieces, ferrule-based	Viper	Two-pieces, ferrule-based	
Zero-dead volume by design	-	-	+	-	Loss of resolution, carryover
Support of UHPLC pressures	-	-/+	+	+	Leakage
Compatible with different vendors' column and valve hardware	+	-/+	+	-	Reduced flexibility, possible leakage or dead volume after replumbing
Fixation of capillary in correct port position	-	-	+	+	Dead volume, leakage, carryover
No wear on receiving ports	+	+	+	-	Leakages, increased maintenance effort and costs
Error-free handling without dedicated user training	+	-	+	-	All issues above

Table 2. Performance Criteria from Standard Test to Compare the Robust Performance of the Viper System Against the Initial Performance of a Conventional Fitting and its Performance After Pressure Stress

Acetanilide	Conventional Finger-tight Fitting (Optimally mounted)	Conventional Fingertight Fitting (Slipped)	Viper Fingertight Fitting	Viper vs. Optimal Conventional	Viper vs. Slipped Conventional
Plates [N]	1112	998	1405	+ 26%	+ 41%
Peak Height [mAU]	349.8	308.1	392.5	+ 12%	+ 27%
Resolution [EP]	5.0	4.7	5.6	+ 12%	+ 19%

Table 3. Efficiency Data Comparison Between Viper Plumbing and Conventional Plumbing for All Retained Peaks					
n = 6	Theoretical Plates [EP]				
	Conventional Capillary and Fitting Setup w/o Valves [Mean ± SD]	Viper Setup w/o Column Switching Valves [Mean ± SD]	Viper Application Switching Setup [Mean ± SD]	Viper w/o Valves vs. Conventional w/o Valves [%]	Viper Application Switching vs. Conventional w/o Valves [%]
Naphtalene	14.014 ± 119	16.086 ± 18	15.743 ± 104	+ 14.8	+ 12.3
Biphenyl	14.890 ± 79	16.355 ± 67	15.957 ± 18	+ 9.7	+ 7.2
Fluorene	15.086 ± 112	16.294 ± 68	16.129 ± 66	+ 8.0	+ 6.9
Anthracene	15.474 ± 132	16.443 ± 38	16.433 ± 41	+ 6.3	+ 6.2
Fluoranthene	15.127 ± 103	15.878 ± 54	15.990 ± 54	+ 5.0	+ 5.7

Hardware Setup A

SRD-3400 Solvent Rack with 4 degasser channels

HPG-3400RS Rapid Separation Binary Pump with 2 solvent selector valves

WPS-3000TRS Rapid Separation Well Plate Sampler, thermostatted

TCC-3000RS Rapid Separation Thermostatted Column Compartment

VWD-3400RS Rapid Separation Variable Wavelength Detector

Semi-micro flow cell for VWD-3x00, SST, 2.5 µL, 7 mm

Method Conditions A

Separation Column: Acclaim® RSLC 120, C18, 2.2 µm, 2.1 × 50 mm, Dionex P/N 068981

Mobile Phase A: Water, in-house HPLC quality

Mobile Phase B: Acetonitrile, ultra-gradient HPLC grade, Baker P/N 9017

Flow Rate: 2.6 mL/min

Elution Conditions: Isocratic, premixed, 50% A: 50% B

Sample Temperature: 20 °C

Sample Volume: 1 µL

Sample Mixture: Alkylphenone standard with 9 alkylphenones and Uracil

Column Temperature: 35 °C

Wavelength: 254 nm

Data Collection Rate: 25 Hz

Time Constant: 0.06 s

Hardware Setup B

SRD-3600 Solvent Rack with 6 degasser channels

DGP-3600RS Rapid Separation Dual-Gradient Pump

WPS-3000TRS Rapid Separation Well Plate Sampler, thermostatted

TCC-3000RS Rapid Separation Thermostatted Column Compartment with external switching valves

Left valve: 2-position 6-port valve, type HT, for pressures up to 1,000 bar

Right valve: 2-position 10-port valve, type HT, for pressures up to 1,000 bar

DAD-3000RS Rapid Separation Diode Array Detector

Semi-micro flow cell for DAD-3000, SST, 2.5 µL, 7 mm

Method Conditions B

Separation Column: Acclaim 120, C18, 3 µm, 2.1 × 150 mm, Dionex P/N 059130

Mobile Phase A: Water, in-house HPLC quality

Mobile Phase B: Acetonitrile, ultra gradient HPLC grade, Baker P/N 9017

Flow Rate: 0.67 mL/min

Elution Conditions: Isocratic, dial-a-mix, 35% A: 65% B

Sample Temperature: 25 °C

Sample Volume: 1 µL

Sample Mixture: PAH standard with 5 PAHs and Uracil

Column Temperature: 30 °C

Wavelength: 251 nm

Data Collection Rate: 50 Hz

Slit Width: Wide

Response Time: 0.1 s

RESULTS AND DISCUSSION

Assessment of Viper for Robust Zero-Dead-Volume UHPLC Plumbing (Setup A)

With Setup A, a conventional one-piece fingertight fitting system was compared against the Viper fitting at a backpressure of approximately 600 bar during long-term operation with a test mix. A mixture of nine alkylphenones was injected consecutively with both the conventional and the Viper fitting installed at column inlet and outlet, until a first change in chromatographic performance became visible (Figure 3).

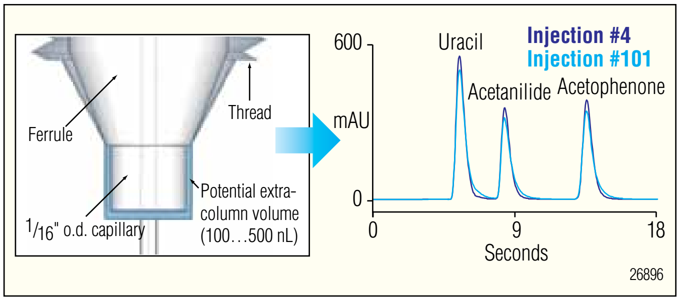

Figure 3. Conventional fitting systems often create extracolumn volumes caused by incorrect positioning of the ferrule or by slipping of the capillary when subjected to high pressures. The chromatogram demonstrates deteriorated peak shape caused by a slipped capillary at a backpressure of only 600 bar (8,700 psi).

After approximately 100 injections, the one-piece fingertight fitting at the column inlet began to slip (confirmed by inspection), which resulted in increased precolumn extracolumn volume. Figure 3 demonstrates the potential dead volume in a conventional fluidic connection and the effects on the chromatogram (right). Detailed results are given in Table 2.

The same experiment was repeated using Viper capillaries and fittings at column inlet and outlet. The results are summarized in Figure 4. When the conventional fitting started to slip under the given operational conditions, the Viper design still ensured robust and reproducible chromatography. Table 2 compares the relevant parameters of peak 2 (acetanilide). Theoretical plates of peaks with small retention factors are typically the most sensitive measure for extracolumn band-broadening effects. It can be seen that after 100 runs the Viper system yielded more than 40% higher efficiency than the conventional fitting. The data on resolution and peak height reflect the performance decline with conventional fittings accordingly.

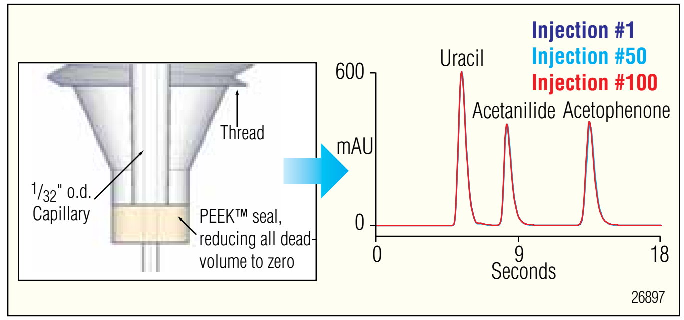

Figure 4. The Viper fingertight fitting system does not use a ferrule and eliminates any extracolumn volumes by design. The chromatogram overlay shows consistent peak shapes under identical conditions as in Figure 3.

In addition to Figures 3 and 4, Figure 5 compares all three peaks for differences in system efficiency, when switching from Viper to a conventional fitting for both before and after long-term pressure stress.

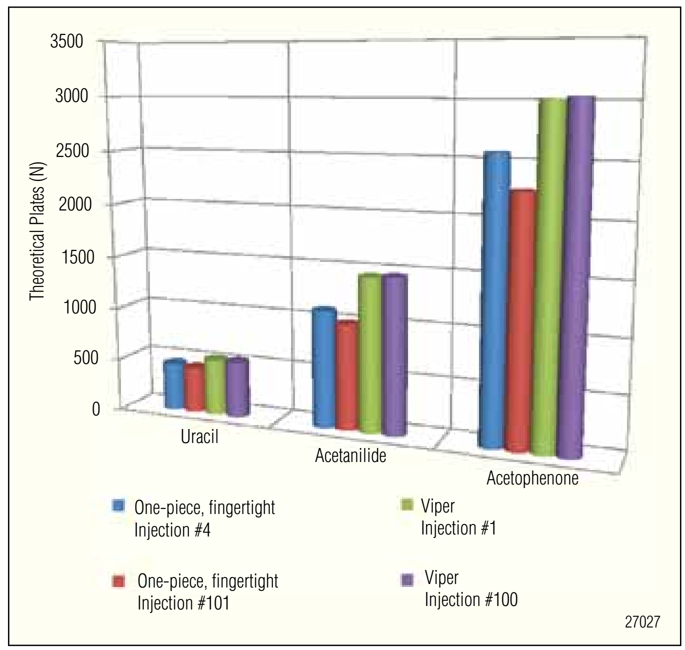

Figure 5. Differences in efficiencies with Viper vs. conventional fingertight fitting before and after long-term pressure stress.

Assessment of the Viper System for Easy Plumbing of Advanced Chromatographic Schemes (Setup B)

The separation column is typically the fluidic part of an HPLC system which experiences the most frequent and regular reconnections. Nevertheless, there is also an increasing request for advanced column switching techniques to boost instrumental capabilities. These fluidic schemes bear the risk of performance losses due to the use of switching valves and additional fitting connections. It is crucial to reduce such performance losses to a minimum to yield significant profitability with these advanced techniques. Moreover, their setup should require as little special operator skill as possible to improve method ruggedness.

The experiments performed with setup B compare the influences of switching valves and different fitting systems on chromatographic performance with the example of a distinct advanced setup for automated application switching (Figure 6). To provide a data set for comparison similar to setup A, a conventional system without column switching was equipped with state of the art $^{1}/_{16}$" o.d. capillaries at an i.d. of 0.13 mm. The number of theoretical plates was measured, as it the most sensitive performance criterion for extracolumn volume effects.

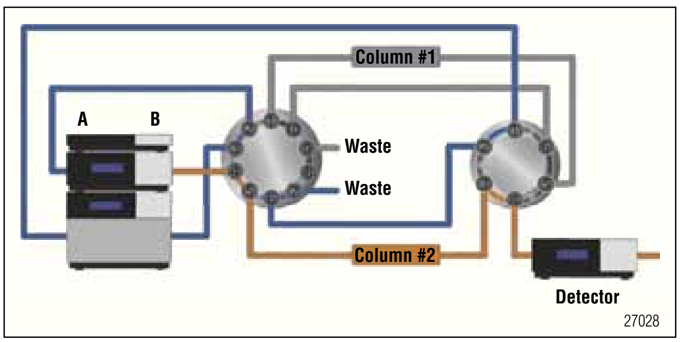

Figure 6. Advanced setup for automated application switching. This configuration employs a total of 555 cm capillary length, two switching valves, and two separation columns and showcases the importance of fluidic connections in LC.

As a next step, the same conventional system was equipped with Viper capillaries and fittings, and this setup was compared against the performance of the automated application switching setup completely connected with Viper capillaries (Figure 7).

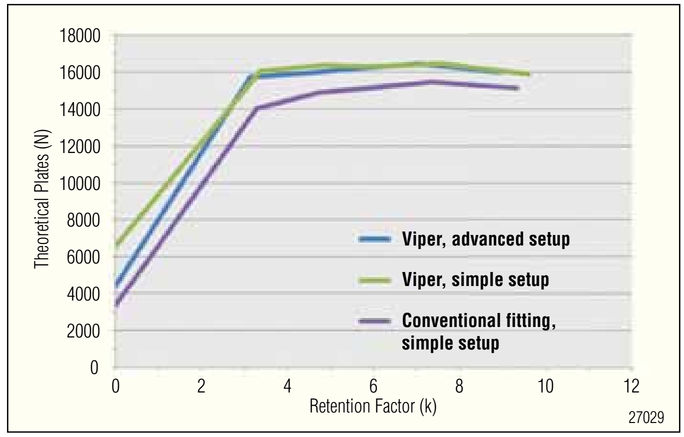

Figure 7. Peak efficiency comparison at different retention factors between simple and advanced Viper plumbing and conventional simple plumbing.

Plotting the measured theoretical plates versus the retention factor (see Figure 7) shows that an advanced column switching setup with two valves and considerable Viper capillary lengths of several meters still performs markedly better than a standard system setup with conventional fittings and without additional valves. Considering the plate numbers at retention factors of approximately 3, it provides more than 12% higher efficiencies (Table 3). The efficiency drop between a standard setup to an application switching setup was negligible as long as Viper capillaries were used. Even with the advanced application switching setup, the use of the Viper system provides 6 –12% better performance over the simple standard setup at comparable conditions. Table 3 shows the related data for the peaks of all retained components.

General Assessment on Flexibility and Ease of Use of Viper

Advanced column switching techniques can significantly boost productivity of analytical laboratories. At the same time, one has to rely on zero-dead-volume connections at the installed switching valves. Using conventional fittings, error-free installation especially at 10-port valves requires skilled users, a dedicated wrench tool, and is time consuming. As depicted in Figure 8, the integrated and removable knurl of the Viper fitting supports its use, even in space-challenging situations. Together with its easy and intuitive handling, complete and guaranteed zero-dead volume, plumbing of such a valve can be completed in < 5 min.

Figure 8. A 10-port valve completely connected with Viper capillaries. Note that the black knurl can be removed easily after plumbing.

Automated column scouting is a powerful tool for ultrafast LC method development. Figure 9 shows a thermostatted column compartment with six columns that can be automatically screened employing the two integrated 6-position 7-port valves. In method development, separation scientists are confronted with frequent reconnections of columns from different vendors with different port designs. The Viper system proved to be the ideal fitting system for this purpose, as it ensures proper connection and reconnection to any tested vendor's column hardware. At the same time, it is very small in size and thus supports column lengths exploiting the total internal width of the thermostatted column compartment.

Figure 9. Viper-equipped column thermostat for automated switching between six LC columns in unattended method development.

SUMMARY

The recently introduced Viper fingertight fitting system shows improved chromatographic results when compared with conventional capillary and fitting systems. Even advanced setups with multiple switching valves and use with LC hardware are possible without any special attention or user training.

The key values of the Viper fingertight fitting system are:

- Guaranteed zero-dead volume connections by design
- Highest possible ease of use for robust multiple reconnections
- Highest possible performance, guaranteed even for advanced column switching setups
- Highest flexibility through compatiblity with virtually any common column and valve type

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