TECHNICAL NOTE 74068

Increase sensitivity in UHPLC-MS analysis by minimizing post-column dispersion

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Goal

Reduce post-column dispersion with small ID capillaries for fast gradient UHPLC-MS methods using sub-2 µm particle next-generation columns

Introduction

The Thermo Scientific™ Vanquish™ Horizon UHPLC system delivers unrivaled performance and throughput, making it the instrument of choice for applications requiring high-end UHPLC. The system supports the use of all commercially available analytical-flow column dimensions and particle sizes and can deliver flow rates up to 5 mL/min.[1] To accommodate the use of different column formats and flow rates, the fluidic set-up of the system was designed to reduce extra-column band dispersion while allowing for only moderate system pressure even at elevated flow rates.

Extra column volume is defined as the volume from the point of injection to the column inlet, plus the volume from the column outlet to the detector. Extra column volume causes system dispersion, which adds to the inherent column band broadening. The system dispersion (or band broadening) affects various chromatographic parameters in (U)HPLC, including detection sensitivity, resolution, theoretical plate number, and peak capacity.[2] It is desirable to minimize system dispersion, for instance, when the analysis of complex samples using UHPLC-MS requires high peak capacity and high throughput.[3,4] Reducing system dispersion increases the resolution of peaks, and hence, reduces competitive ionization, leading to better MS signal quality. One tool to reduce system dispersion is the use of connection capillaries with narrower internal diameter (ID). In case of gradient separations, reducing the ID of post-column capillaries is usually sufficient for the optimization due to the peak compression effect of the gradient on the column. However, the use of smaller ID capillaries results in additional back pressure. For fast methods utilizing state-of-the-art columns with small particle sizes and thus demanding high optimal linear velocities, the maximum available pressure of a UHPLC

system can often be insufficient to cope with the additional back pressure caused by the narrow capillaries, and hence, fluidic optimization becomes unfeasible. The Vanquish Horizon UHPLC system benefits from its high upper pressure limit of 1,500 bar (22,000 psi), allowing the use of narrow capillaries to minimize system dispersion, even when pressures exceed the capabilities of the majority of modern UHPLC systems.

This technical note demonstrates an easy and cost-efficient optimization of post-column dispersion in a UHPLC-MS system, where system capillaries are simply replaced between the column and the detector. The effects of the system capillaries on post-column dispersion are evaluated by peak width, peak height, and peak capacity, using the Vanquish Horizon UHPLC system coupled to the Thermo Scientific™ ISQ™ EM single quadrupole mass detector. A Thermo Scientific™ Accucore™ Vanquish™ C18+ (2.1 mm ID x 100 mm long) column was used. This column is packed with 1.5 µm solid core particles. This sub-2 µm next-generation particle has an ultra-short diffusion path, allowing highly efficient and fast separations for complex mixtures at operating pressures up to 1,500 bar. The optimized configuration, employing small ID 75 µm Thermo Scientific™ nanoViper™ Fingertight fittings, is ideal for high-throughput UHPLC-MS analyses that require high peak capacity.

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm at 25 °C, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System (P/N 50136149)
- Methanol, Optima™ LC/MS grade, Fisher Chemical™ (P/N A456-212)
- Formic acid, Optima™ LC/MS grade, Fisher Chemical™ (P/N A117)
- Ammonium formate, Optima™ LC/MS grade, Fisher Chemical™ (P/N A115)
- Boscalid, Carbendazim, Carbofuran, Diuron, Fluometuron, Metazachlor, Mepiquat chloride, Naptalam, Quinoxyfen, Uniconazole, Analytical standard grade. Purchased from reputable vendors.

Sample handling

- Fisher Scientific™ Fisherbrand™ Mini Vortex Mixer (P/N 14-955-152)
- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes:
  - 100–1000 µL (P/N 4641100N)
  - 10–100 µL (P/N 4641070N)
  - 1–10 µL (P/N 4641030N)
- Thermo Scientific™ SUN-SRi™ 12 × 32 mm Standard Opening Crimp Vials (amber, 2 mL) (P/N 22-313375)
- Thermo Scientific™ SUN-SRi™ 11 mm Orange Snap Caps (P/N 14-823-381)

Instrumentation

- Vanquish Horizon UHPLC system consisting of:
  - System Base Vanquish Horizon/Flex (P/N VF-S01-A)
  - Vanquish Binary Pump H (P/N VH-P10-A)
  - Vanquish Split Sampler HT (P/N VH-A10-A)
  - Vanquish Column Compartment H (P/N VH-C10-A-02) with active preheater (P/N 6732.0110)
  - Thermo Scientific™ Viper™ Capillary MP35N Fingertight Fittings, 100 µm × 550 mm (P/N 6042.2360)
  - Thermo Scientific™ nanoViper™ Fingertight Fittings, 75 µm × 550 mm (P/N 6041.5760)
  - Thermo Scientific™ nanoViper™ Fingertight Fittings, 75 µm × 250 mm (P/N 6041.5730)
  - Thermo Scientific ISQ EM Single Quadrupole Mass Spectrometer (P/N ISQEM-ESI)

Sample preparation

Stock solutions of nine pesticide compounds (boscalid, carbofuran, diuron, fluometuron, mepiquat chloride, metazachlor, naptalam, quinoxyfen, and uniconazole) were prepared at a concentration of 1 mg/mL in solvent A (refer to the table *Chromatographic conditions* for more details). The stock solution of carbendazim was prepared at a concentration of 100 µg/mL due to low solubility in solvent A. The stock solutions were stored at -20 °C. Working solutions were prepared freshly at a concentration of 10 µg/mL by diluting the stock solution with an appropriate volume of solvent A.

Chromatographic conditions

Parameter	Value
Column	Accucore Vanquish C18+ (2.1 × 100 mm, 1.5 µm) P/N 27101-102130
Solvents	A: Water containing 5 mM ammonium formate and 0.1% formic acid B: Methanol/Water (95:5, v/v) containing 5 mM ammonium formate and 0.1% formic acid
Gradient	Time (min) %B 0 5 5 95 5.2 5 10 5
Flow rate	0.4 mL/min
Column temp.	40 °C (forced air, active pre-heater at 40 °C)
Sampler temp.	4 °C
Injection volume	0.1 µL

MS detector settings

Parameter	Value
Ionization mode	ESI
Polarity (Spray voltage)	Positive (+3,000 V)
SIM scan	SIM masses are listed in Table 1.
Chrom filter	Off
SIM width	0.1 amu
Dwell time	0.005 s
CID voltage	20 V
Vaporizer temp.	227 °C
Ion transfer tube temp.	300 °C
Gas flow pressure	Sheath gas: 42.9 psig Auxiliary gas: 4.8 psig Sweep gas: 0.0 psig

Table 1. Formula and and SIM mass for ten pesticide compounds. SIM mass is $[M+H]^+$ for all compounds except mepiquat, for which SIM mass is $[M]^+$.

Name	Formula	SIM *m/z*
Mepiquat	$C_7H_{16}N^+$	114.2
Carbendazim	$C_9H_9N_3O_2$	192.1
Carbofuran	$C_{12}H_{15}NO_3$	222.1
Naptalam	$C_{18}H_{13}NO_3$	292.1
Fluometuron	$C_{10}H_{11}F_3N_2O$	233.1
Metazachlor	$C_{14}H_{16}ClN_3O$	278.1
Diuron	$C_9H_{10}C_{l2}N_2O$	233.0
Boscalid	$C_{18}H_{12}C_{l2}N_2O$	343.0
Uniconazole	$C_{15}H_{18}C\,N_3O$	292.1
Quinoxyfen	$C_{15}H_8C_{l2}FNO$	308.0

Chromatography Data System

The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.3 was used for data acquisition and analysis.

Results and discussion

Steep gradients produce extremely narrow peaks that are heavily affected by the extra-column dispersion.[2] Peak compression in gradient elution mitigates the pre-column system dispersion contribution. Thus, fluidic optimization is mostly required to reduce post-column dispersion. For the UHPLC-MS setup used in this work, the column outlet capillary and the ISQ EM fluidics from the instrument inlet to the source were exchanged (see below for more details). The dispersion was estimated by performing a 5-minute gradient run of a ten pesticide standards mixture. At a flow rate of 0.4 mL/min, the total back pressure with optimized tubing exceeded 1,250 bar. A higher flow rate was not feasible with this fluidic setup due to the generated back pressure.

The impact of post-column dispersion was assessed by comparing different fluidic configurations with different ID capillaries. Chromatography parameters, namely peak width, peak height, and peak capacity were monitored for this purpose. Figure 1 shows flow schemes of different configurations in the Vanquish Horizon UHPLC system with the ISQ EM mass detector. The standard post-column configuration for the Vanquish Horizon UHPLC system coupled to an ISQ EM mass detector consists of a Viper Capillary MP35N Fingertight Fitting (ID × L, 100 µm × 550 mm), indicated as line A, two PEEK capillaries (ID × L, 127 µm × 225 mm and 127 µm × 300 mm), indicated as dashed lines, and a PEEK capillary (ID × L, 127 µm × 236 mm), indicated as line C. The Viper capillary (A) is connected to the inlet union of the ISQ EM, and the PEEK capillary (C) connects the grounding union to the spray probe. The PEEK capillaries illustrated as dashed lines connect the instrument inlet to the calibration valve, and the calibration valve to the grounding union.

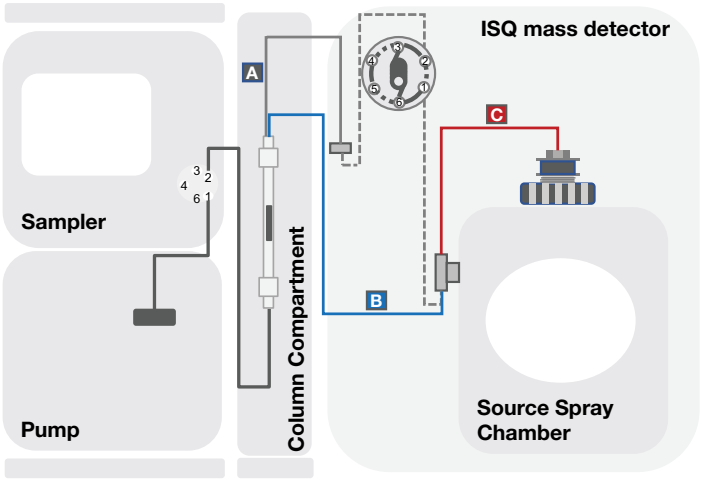

Figure 1. Flow schemes of different configurations in the Vanquish Horizon UHPLC system with ISQ EM mass detector

First, a Viper MP35N capillary (ID x L, 100 µm × 550 mm) in the standard configuration (indicated as line A in Figure 1) was replaced by a nanoViper fitting (ID × L, 75 µm × 550 mm). Figure 2 shows the comparison between the two capillaries with respect to peak height and peak width at half-height. The reduction of capillary ID from 100 µm to 75 µm resulted in a 2% to 14% (average 9%) decrease in peak width at half-height and -2% to 13% (average 8%) increase in peak height. Mepiquat peak height was not affected by the capillary configuration because of the high tailing factor observed under the separation conditions. The peak-shape of mepiquat was not improved by changes of the extra-column volume, pointing to the chemical nature of the tailing. Naptalam was not displayed in Figure 2a due to its relatively small peak height compared to the other nine compounds. The peak height of napatalam increased by 7% with the use of the 75 µm ID capillary. The use of a smaller ID column-outlet capillary reduced system dispersion and resulted in narrower, higher peaks.

Further optimization included both column outlet and ISQ EM source fluidics. The ISQ EM instrument inlet and calibration valve were bypassed by connecting the column outlet directly to the grounding union with a 75 µm × 55 cm nanoViper fitting. Moreover, the default PEEK capillary between grounding union and source (line C in Figure 1) was replaced by a 75 µm × 25 cm nanoViper fitting. Figure 3 clearly shows the improvement in both peak height and peak width with the optimized configuration. The peak height with the optimized configuration increased by up to 65% (average 42%), and the peak width at half-height decreased up to 38% (average 29%), compared to the standard configuration. Comparative experiments, shown in Figure 2 and Figure 3, were respectively performed within a day to reduce inter-day variation. The peak heights and widths using 100 µm ID capillary in Figure 2 (blue bars) are slightly different from those using standard configuration in Figure 3 (blue bars), due to the inter-experiment variance. The Vanquish Horizon

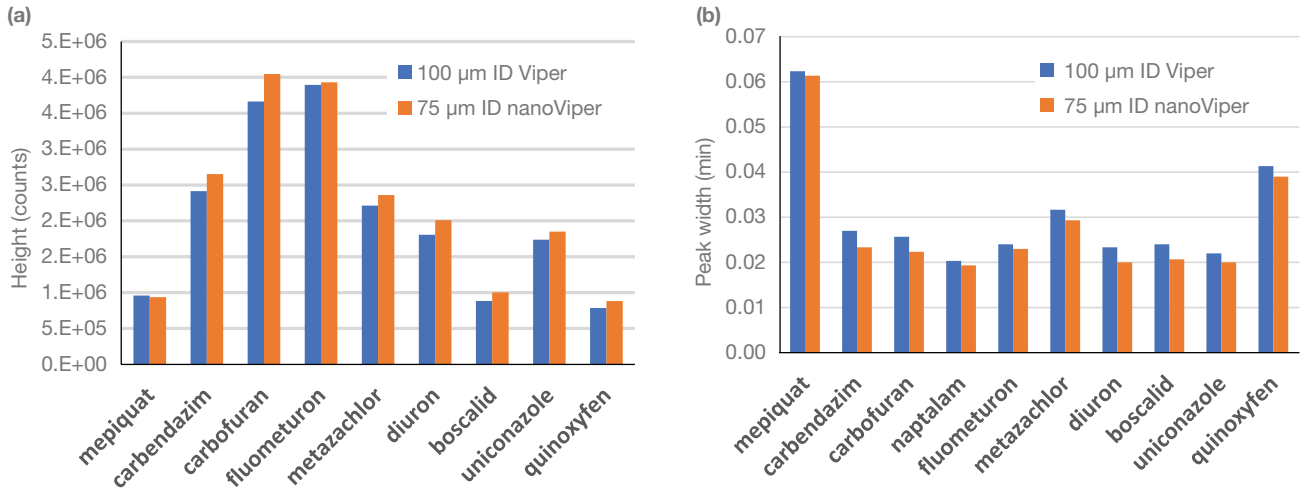

Figure 2. Peak heights (a) and peak widths at half-height (b) with a 5-minute gradient elution using different column-outlet capillaries with standard configuration. The capillaries on line A in Figure 1 were replaced. Blue bars: Viper MP35N (ID × L, 100 µm × 550 mm); Orange bars: nanoViper (ID × L, 75 µm × 550 mm)

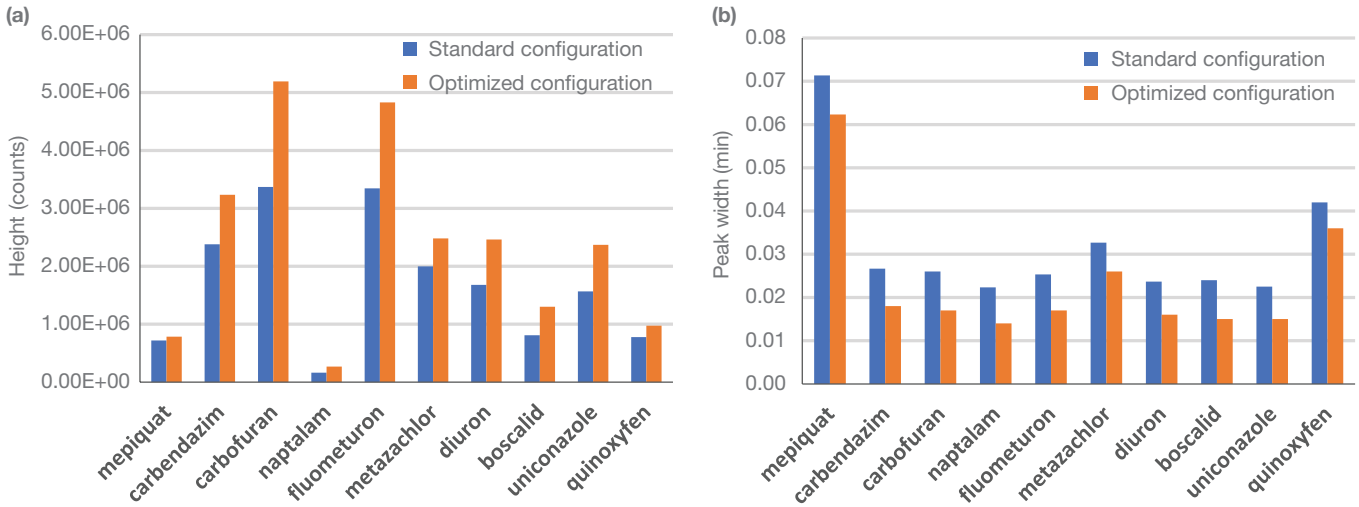

Figure 3. Peak heights (a) and peak widths at half-height (b) with a 5-minute gradient elution before and after optimization of column-outlet and ISQ EM fluidics. Blue bars: standard configuration; Orange bars: optimized configuration

system pressure specification is 1,500 bar, which made this optimization with smaller ID capillaries possible, without reducing flow rates. The maximum system pressure with the optimized configuration was around 1250 bar, versus 1160 bar in the standard configuration.

Table 2 summarizes percentage improvements in peak heights and widths when replacing a 100 µm Viper capillary with a 75 µm nanoViper capillary in Figure 2. In addition, percentage improvements in peak heights and widths with optimized configuration (compared to standard configuration) are listed in Figure 3. Positive and negative values indicate the increase and the decrease in peak height and width, respectively.

Table 2. Improvements in peak heights and widths with both nanoViper compared to Viper capillaries (in Figure 2) and optimized compared to standard configuration (in Figure 3). Positive and negative values indicate the increase and the decrease in peak height and width, respectively.

	75 µm nanoViper vs. 100 µm Viper column outlet capillaries		Optimized vs. standard configurations	
	Peak height (%)	Peak width at half height (%)	Peak height (%)	Peak width at half height (%)
Mepiquat	-2	-2	9	-13
Carbendazim	10	-14	36	-33
Carbofuran	10	-13	54	-35
Naptalam	7	-5	65	-37
Fluometuron	1	-4	44	-33
Metazachlor	7	-7	24	-20
Diuron	11	-14	47	-32
Boscalid	13	-14	61	-38
Uniconazole	6	-9	51	-33
Quinoxyfen	12	-6	25	-14
Average	8	-9	42	-29

Figure 4 illustrates examples of chromatograms of five selected compounds (i.e., boscalid, carbendazim, fluometuron and diuron, and naptalam), analyzed with the standard configuration (black chromatogram) and with the optimized configuration (red chromatogram). The peaks obtained with the optimized configuration are narrower and of higher intensity compared to those with the standard configuration due to the reduced post-column dispersion in the optimized configuration.

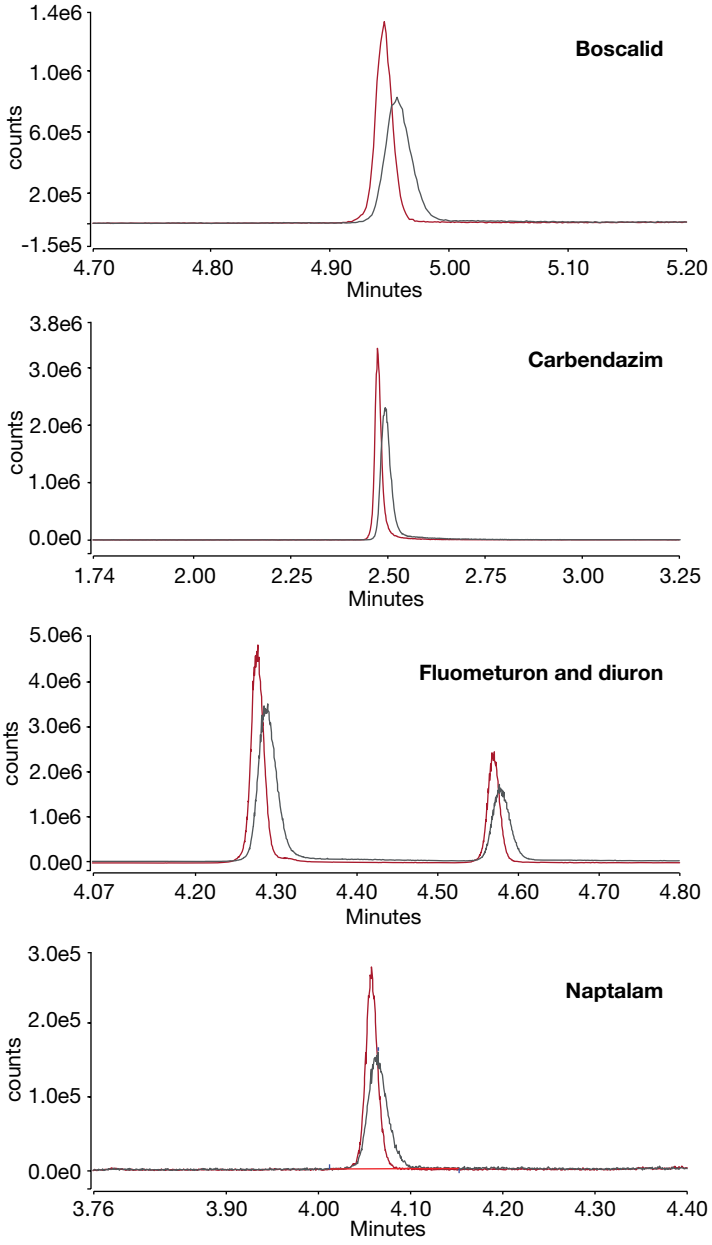

Figure 4. SIM chromatograms of five compounds (boscalid, carbendazim, fluometuron and diuron, and naptalam), showing higher intensity and narrower peaks using the optimized configuration compared to the standard configuration

Peak capacity using different capillary configurations was evaluated to assess the impact of tubing diameter on resolution. Peak capacity, the most common measure of separation power in gradient elution, was calculated based on the average peak width at 4σ and the retention window (i.e., the retention time difference between the first and last peak in a chromatogram) by the following Equation:

$$n_c = 1 + \frac{t_L - t_F}{w_{4\sigma}}$$

where t_L and t_F is the retention time of the last and first peak, respectively, and $w_{4\sigma}$ is the averaged value of peak width at 4σ for all the ten compounds.[4]

The use of the optimized configuration in the system provided 36% higher peak capacity compared to the standard configuration, for a short 5-minute gradient run on the 2.1 x 100 mm column packed with 1.5 µm particles (Figure 5). The result demonstrates that capillary optimization improves the resolving power of methods based on fast gradients.

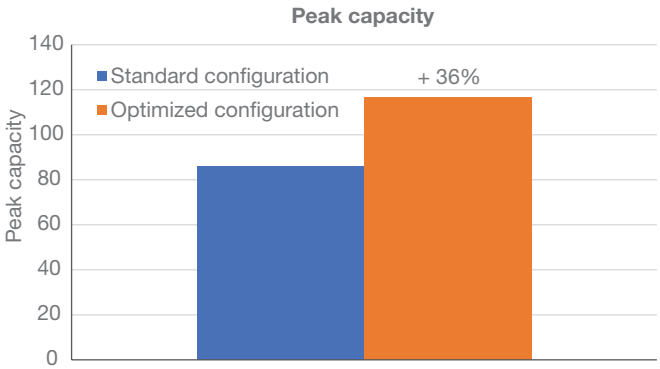

Figure 5. Comparison of peak capacity for a 5-minute gradient on a 2.1 × 100 mm column packed with 1.5 µm particles with standard- and optimized configurations

Conclusion

- Extra-low system dispersion required in fast-gradient methods using sub-2 µm particle next-generation columns can be achieved with the Vanquish Horizon UHPLC system by optimization of the post-column fluidics.
- The unique pressure specification of the Vanquish Horizon UHPLC system allows the replacement of default tubing with 75 µm capillaries without risking the system overpressuring.
- Post-column fluidics optimization resulted in up to 65% increase in peak height, up to 38% reduction of peak width at half-height, and a 36% increase in peak capacity compared to the standard configuration.

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