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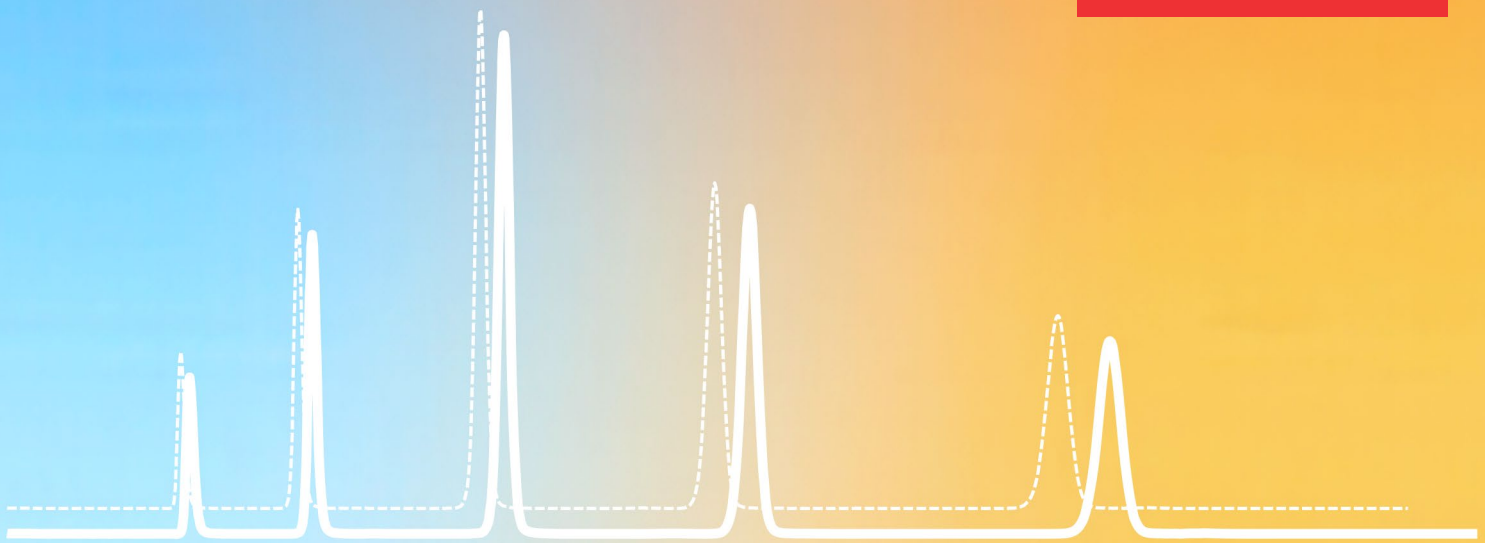

HPLC

Effective mobile phase pre-heating: Why it matters and how it is achieved

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Keywords

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Introduction

The separation in liquid chromatography (LC) is usually discussed in terms of the impact of stationary phase chemistry, mobile phase composition, flow rate, and gradient profile. Comparatively less attention is paid to the thermal control of the column and especially of the mobile phase entering it, even though these can have similarly critical influences on the chromatographic results. Moderate differences in effective internal column temperature can already have substantial effects on solvent viscosity, system backpressure, analyte diffusion and adsorption, and directly translate into shifts in retention time, selectivity, and peak shape.[1] In common practice, most high-performance liquid chromatography (HPLC) systems are equipped with a column oven or column thermostat, which is typically used to keep the column at a stable temperature and prevent temperature-related run-to-run and instrument-to-instrument variability. However, the column temperature is not only affected by its external thermal conditions but also significantly by interior conditions, in particular, the temperature and flow of the incoming mobile phase, or ultra-high pressure conditions that could cause frictional heating. With increasing flow rate and delta between column and mobile phase temperature, the chances that chromatographic performance is impacted by the mismatch also increase. Mobile phase pre-heating addresses this gap by conditioning the eluent to the intended temperature before it enters the column.

Modern LC platforms, including the Thermo Scientific™ Vanquish™ HPLC and UHPLC Systems, offer multiple active or passive pre-heater designs, allowing users to tailor temperature management to their application needs. This white paper provides practical guidance for selecting and applying these options to achieve stable separations with appropriate run-to-run and day-to-day repeatability. In addition, establishing accurate and well-documented temperature control during method development can substantially simplify method transfer between instruments and laboratories by reducing temperature-driven variability.[2]

Why eluent thermostatting matters: Thermal mismatch

Temperatures above (or sometimes below) ambient are typically used in HPLC and UHPLC to optimize the chromatographic separation.[1] In most cases, retention times decrease with increasing temperature, which decreases the overall run time of a method and narrows the eluting peaks. In addition, the lower mobile phase viscosity at elevated temperatures allows for higher flow rates to be used within a given pressure limit, which can further accommodate the frequent need for quick separations. As the effect on retention is not the same for each molecule but, according to the Van't-Hoff equation, differs depending on substance-specific properties, temperature adjustments can be successfully implemented to tweak the separation selectivity.[3] These effects on selectivity, efficiency, and thus resolution of a method are determined by the **effective** temperature inside the column and, if applicable, even its local distribution.

From its various influencing factors, the column oven setpoint is just the most obvious one, but the temperature and flow rate of the mobile phase that enters the column and the thermostatting principle are equally relevant. At pressure rates above 400 bar (UHPLC conditions), heat created by viscous friction between mobile and stationary phase and its preservation or dissipation can play an additional role.[1,2]

The consequences of a thermal mismatch of column and mobile phase are depicted in Figure 1. Due to the very slow heat exchange in quasi-adiabatic column thermostats (e.g., still air thermostats, Figure 1A) these can hardly counteract the cooling effect of a colder mobile phase entering the column.[1] The separation happens mostly at the mobile phase temperature, resulting in later retention times than expected from the higher column oven setpoint. Forced air or block heater column ovens show quasi-isothermal behavior (Figure 1B). Hence, colder incoming mobile phase mainly causes lower temperatures in the center of the column, while the column walls are re-heated quickly. The average separation temperature is between the mobile phase and column oven temperatures, but the radial temperature gradient within the column causes peak broadening or distortion because of the different viscosities, flow rates, and separation dynamics at the column center and wall. In an ideal case, the mobile phase temperature is matched with the column temperature by a pre-heating device to achieve the fastest and most efficient separation (Figure 1C).

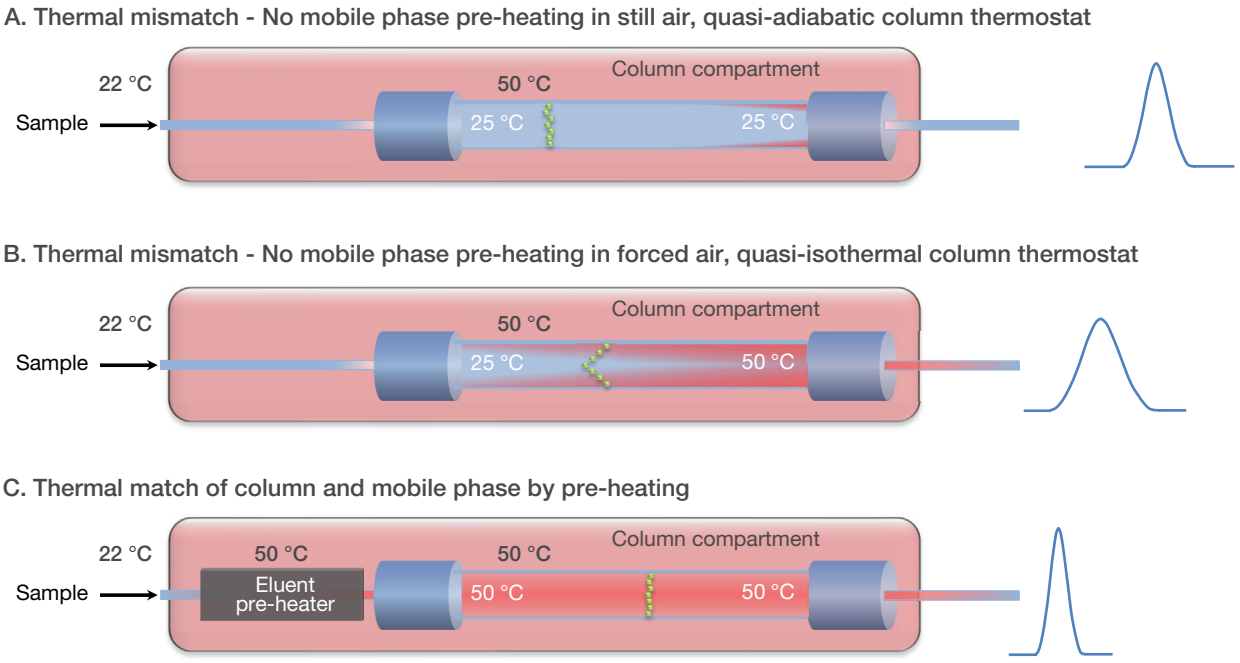

Figure 1. Effect of thermal match and mismatch of column and mobile phase on the temperature distribution inside the column and on chromatographic results.

Different pre-heating options: Active and passive designs

Two different functional principles of mobile phase pre-heaters exist: active and passive ones. Passive pre-heaters are designed as a piece of system tubing, which is in mechanical contact to a temperature-controlled (usually metal) surface inside the column thermostat. The temperature of the incoming eluent is adapted to the column oven temperature by heat exchange with that surface.

Passive pre-heaters:

- Are available from every LC instrument vendor, usually in different internal volume sizes at moderate costs.
- Cannot be controlled independently; they are constrained to the column oven temperature and share its temperature specifications.
- Require fixed mounting to ensure solid contact and heat transfer.
- Can be used for eluent pre-heating and pre-cooling (for separations conducted below ambient temperatures in column compartments with cooling capabilities).

In comparison, active temperature control is achieved by an internal heating element directly mounted to the tubing at the column head. This small electric device is typically designed as a resistance heater and provides independent temperature control of the passing mobile phase.

Active pre-heaters:

- Are offered by few manufacturers only.
- Have higher acquisition costs due to the integrated temperature control device and temperature sensor; however, maintenance costs can be significantly reduced by a dismountable capillary design.
- Provide efficient and controllable eluent heating within autonomous specifications independent of the column thermostat temperature.
- Require electrical contact but can be positioned more flexibly.
- Cannot be used for eluent pre-cooling.

Table 1 and Figure 2 give an overview of pre-heater types available for the Vanquish HPLC and UHPLC systems, all of which come with the recent Thermo Scientific™ Viper™ TQ UHPLC Fittings for simplified, tool-free fluidic connections. As shown in Table 1, the pre-heater models differ not only in their pre-heating performance, which will be reviewed in the following section, but also in physical parameters such as wetted materials, tubing diameter, heated volume, total volume, and extra-column dispersion contribution, all of which can be critical to the success of the chromatographic method.

Table 1. Selection of pre-heaters available for Vanquish HPLC and UHPLC systems with Viper TQ UHPLC fittings.

Product	Abbreviation	Cat. No.	Wetted material	Heated volume	Total volume[a]	System dispersion volume[b]
Thermo Scientific™ Active Pre-heater TQ 0.1 × 380 mm	APH	6732.0700	MP35N, PEK	0.5 µL	3.5 µL	1.65 µL[c]
Thermo Scientific™ Passive Pre-heater TQ 1 µL, 0.1 × 580 mm	PPH-1 µL	6732.0760	MP35N, PEK	1 µL	5 µL	1.83 µL[c]
Thermo Scientific™ Passive Pre-heater TQ 3 µL, 0.18 × 580 mm	PPH-3 µL	6732.0740	SST, PEK	3 µL	17 µL	4.69 µL[d]
Thermo Scientific™ Passive Pre-heater TQ 5 µL, 0.25 × 580 mm	PPH-5 µL	6732.0750	SST, PEK	5 µL	32 µL	8.37 µL[d]

[a] Nominal total volume of pre-heater

[b] System dispersion volumes of complete default system tubing sets were experimentally determined[4] by plotting the total peak variance as a function of the squared retention volume of five isocratically eluting analyte peaks

[c] With Thermo Scientific™ Vanquish™ Flex UHPLC System equipped with Thermo Scientific™ Vanquish™ Diode Array Detector FG with 2.5 µL, 7 mm flow cell and Viper TQ 0.1 × 350 mm MP35N detector inlet capillary

[d] With Thermo Scientific™ Vanquish™ Core HPLC System equipped with Thermo Scientific™ Vanquish™ Diode Array Detector CG with 2.5 µL, 7 mm flow cell and Viper TQ 0.13 × 350 mm SST detector inlet capillary

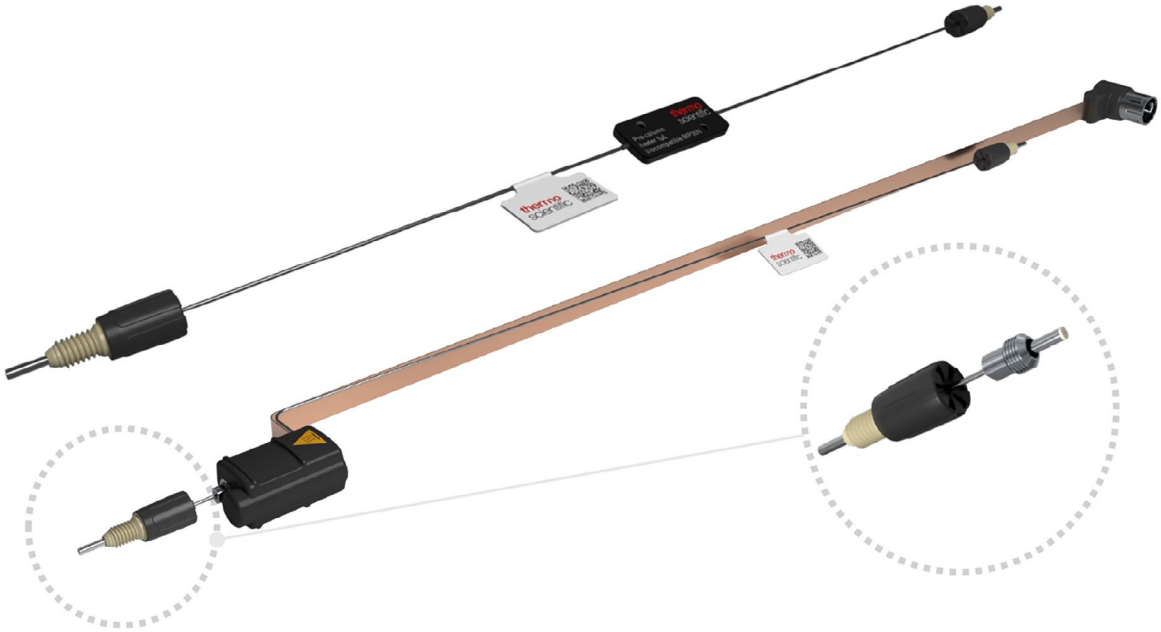

Figure 2. Overview of different pre-heater designs. Passive pre-heater TQ (top) and Active pre-heater TQ (bottom) featuring a replaceable outlet capillary (insert).

Which pre-heater under which conditions? Effective eluent temperature and dispersion trade-offs

To showcase the performance of the pre-heaters listed in Table 1, the effective mobile phase temperature (T_{eMP}) was investigated for each device under various conditions. Each pre-heater was installed on a Vanquish Flex UHPLC system in a Thermo Scientific™ Vanquish™ Column Compartment and fluidically connected to a T-piece. The remaining two ports of the T-piece were connected to a restriction capillary and a temperature probe with a customized Viper fitting so that the temperature of the mobile phase was recorded directly at the outlet of the pre-heater capillary inside the T-piece bore (Figure 3). Nominal temperature setpoints of 30, 40, 60, 80, 100, and 120 °C (equal for column oven and APH) and flow rates of 0.3, 0.6, 1.0, 2.5, and 5.0 mL/min were examined under still air conditions with different mobile phase compositions (containing water, acetonitrile (ACN) or methanol (MeOH)) stored at ambient temperature.

The progression of the T_{eMP} with increasing flow rate is displayed in Figure 4 for several temperature settings and water as the mobile phase for the APH and the three PPH designs. Most conditions indicated a clear advantage of the APH over the PPHs with the T_{eMP} being closer to the setpoint than with the PPHs. In the tested temperature range, all deviations of the T_{eMP} from the setpoint were reasonable with flow rates up to 1 mL/min. For the PPHs, the T_{eMP} started to significantly drop below the column oven setpoint with flow rates above 1 mL/min. The heating performance of the APH is specified with 80 °C at 2.5 mL/min and 120 °C at 1 mL/min. Within these limits, the APH results were well in line with the target temperatures so that the temperature accuracy specification of ±2 K (up to 80 °C) was met. Beyond these specifications, when the heating capacities of the small heating element at the APH were surpassed, the T_{eMP} decreased rapidly. Here the decline was steeper than with the PPHs, so that under extreme conditions, like 120 °C at 2.5 mL/min, the T_{eMP} was higher with the PPHs than with the APH. In these rare high-temperature/high-flow cases and for temperatures below ambient, the PPHs were beneficial over the APH.

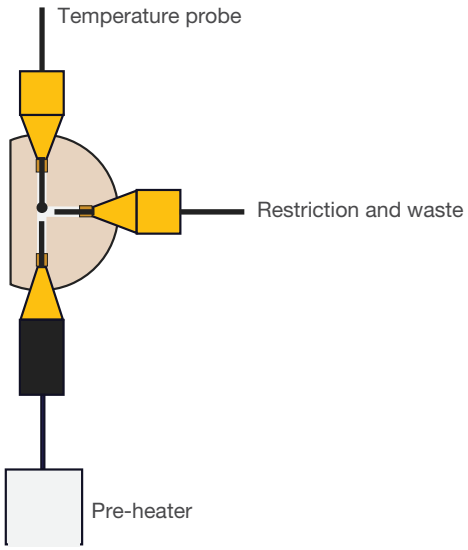

Figure 3. Schematic of experimental setup for T_{eMP} determination.

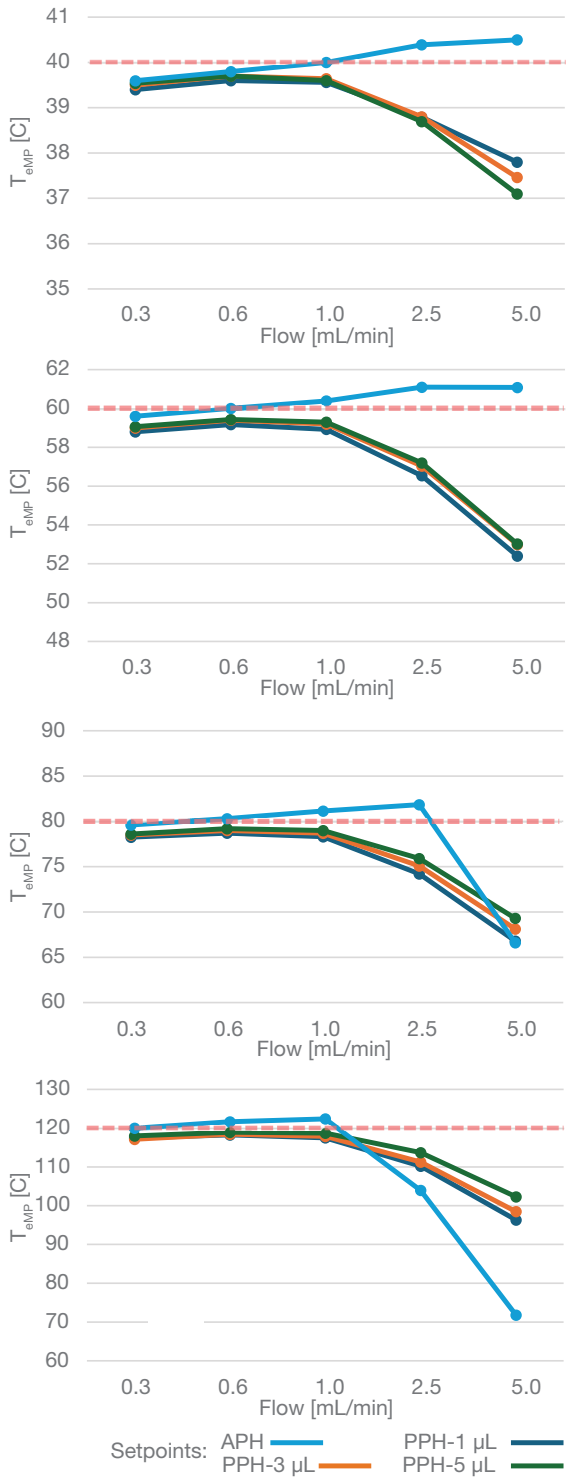

Figure 4. Plots of T_{eMP} achieved with the setup according to Figure 3 for four different pre-heaters at nominal setpoints 40, 60, 80, and 120 °C with water as mobile phase.

The differences between the PPH versions of different volumes were mostly insignificant in the flow range up to 1 mL/min and became a little more evident with increased flow and temperature settings. Slightly higher values of T_{eMP} were typically achieved with increasing nominal heated volume, which can be explained by an improved heating effect that is associated with an increased time span the solvent spends at a given flow in a larger heated volume. For the selection of the best suited PPH geometry for an application, however, other properties than the moderately differing pre-heating performance might be more crucial.[2] Increasing the heated volume requires wider capillary inner diameters, which substantially increases the extra-column volumes of the PPHs (Table 1). Larger system volumes in front of the column can be beneficial in cases when a solvent mismatch of injected sample plug and mobile phase is encountered to improve peak shapes by optimized mixing before the column entry. On the other hand, the interrelated increase in system dispersion can negatively affect peak shapes and efficiency of a method, especially with small diameter columns.[2]

It is important to note that the efficiency of the pre-heating process depends not only on the device itself but also on the physical properties of the eluent, like its thermal conductivity, heat capacity, and heat transfer coefficient. Figure 5 summarizes the T_{eMP} obtained by the APH for three different solvent compositions—water, MeOH/water (50/50, v/v), and ACN/water (70/30, v/v)—beyond its specification limits, at the highest flow rate of 5 mL/min and high setpoints. There is clear evidence that the transfer of heat into the eluent is improved with increasing content in organic solvent of the mobile phase. A comparison of active and passive pre-heating is shown in Figure 6. For the PPH-1 µL, a difference in the T_{eMP} of pure water and 70% ACN was already noticeable from 40 °C upward and amplified with increasing temperature. Again, within its specification limits, the APH was superior to the PPH-1 µL, as stable values of T_{eMP} were recorded, and no significant differences were seen for the eluent compositions. Beyond these limits, the effect of mobile phase change became distinct and the drop of the T_{eMP} with high flows and high temperatures was less pronounced with a high ACN ratio. These observations are particularly relevant in gradient elution methods, where solvent composition changes may lead to variations in pre-heating efficiency depending on the applied pre-heater design.

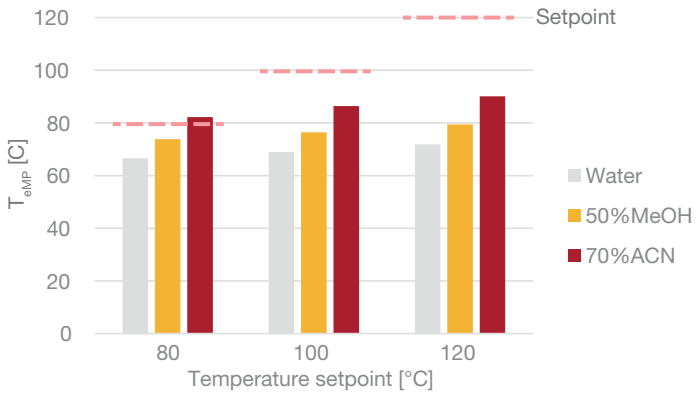

Figure 5. T_{eMP} plot of APH performance versus solvent composition at a flow rate of 5 mL/min. The temperature setpoint exceeds intentionally the specification limit for this flow rate to demonstrate the impact of solvent composition.

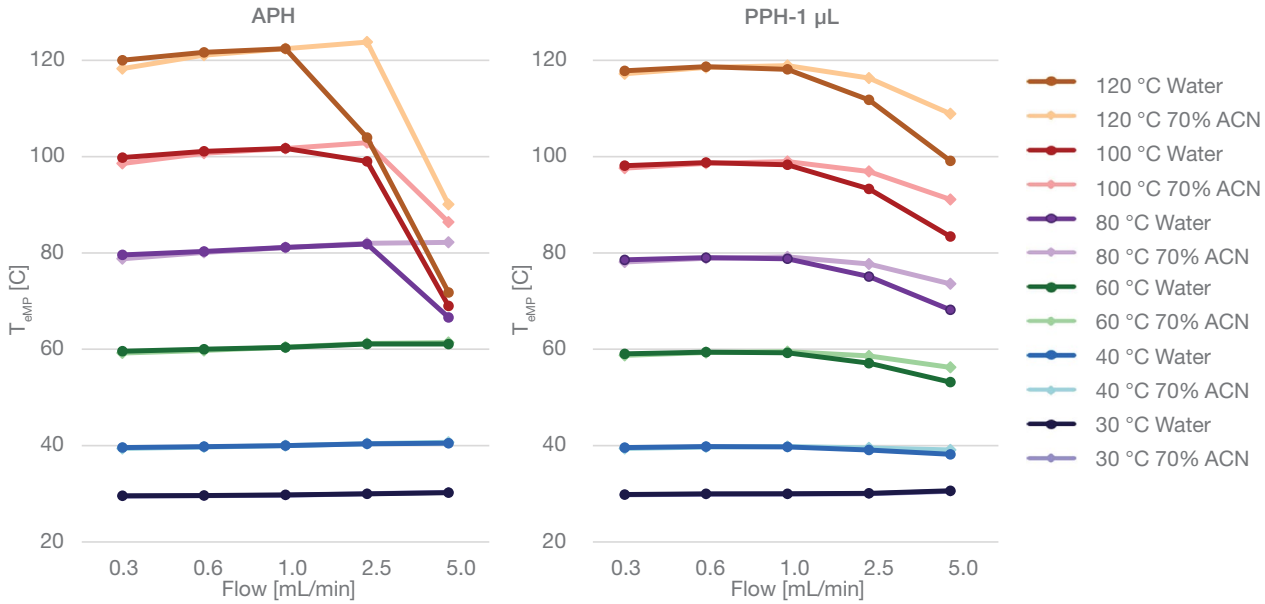

Figure 6. Plots of T_{eMP} of APH (left) and PPH-1 µL (right) at different temperature setpoints with mobile phases water (circles) and ACN/water (70/30, v/v, diamonds). Within its specification limits (80 °C at 2.5 mL·min^{-1}; 120 °C at 1 mL·min^{-1}), the APH exhibited superior heat-transfer characteristics.

Practical impact in chromatography: Examples and recommendations

Some examples for the impact of mobile phase pre-heating in practice are given in Figures 7 and 8. The method for the separation of the active pharmaceutical ingredient (API) mebendazole and its impurities was first applied with a Vanquish Flex UHPLC system as given in the European Pharmacopoeia monograph[5] as a conventional HPLC method (Figure 7) with none, active, and passive eluent pre-heating. Afterwards, it was transferred within the allowable modifications to a smaller diameter column (UHPLC speed-up method, Figure 8). When no pre-heating was applied, a standard Viper capillary 0.1 × 350 mm was installed to connect the autosampler valve and the column.

With the original method, the separation results with APH and PPH-1 µL were very similar (Figure 7). Only slightly later retention times were achieved with the PPH-1 µL due to the slightly lower heating performance, as indicated by Figure 4. Without any pre-heating, however, the separation performance was distinctly affected, with later peak elution due to a lower average column temperature and peak broadening caused by a radial temperature gradient within the column.

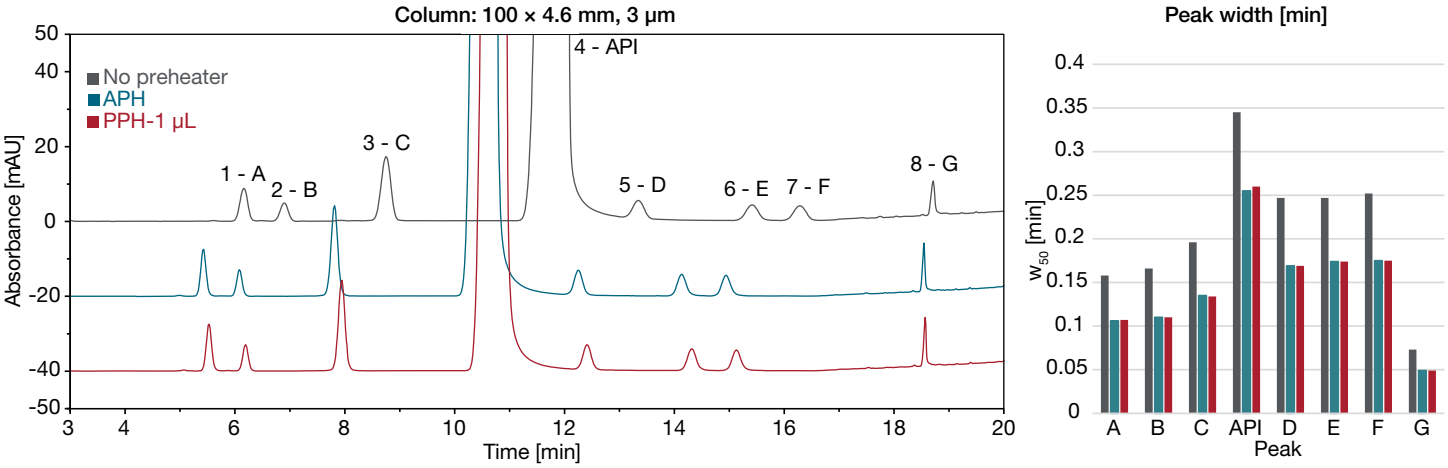

Figure 7. Separation results of mebendazole (API) and impurities (A-G) according to Ph. Eur monograph[5] method without mobile phase pre-heating (gray), with APH installed (teal), and with PPH-1 µL installed (red). Stationary phase: Thermo Scientific™ Hypersil GOLD™ Column, 100 × 4.6 mm, 3 µm (Cat. No. 25003-104630). Mobile phase A: 7.5 g/L ammonium acetate in water; mobile phase B: ACN. Flow rate: 1.2 mL/min. Column temperature: 40 °C (forced air). Gradient: 0 min 20% B – 15.0 min 30% B – 20.0 min 90% B – 25.0 min 90% B – 25.1 min 20% B – 30.0 min 20% B.

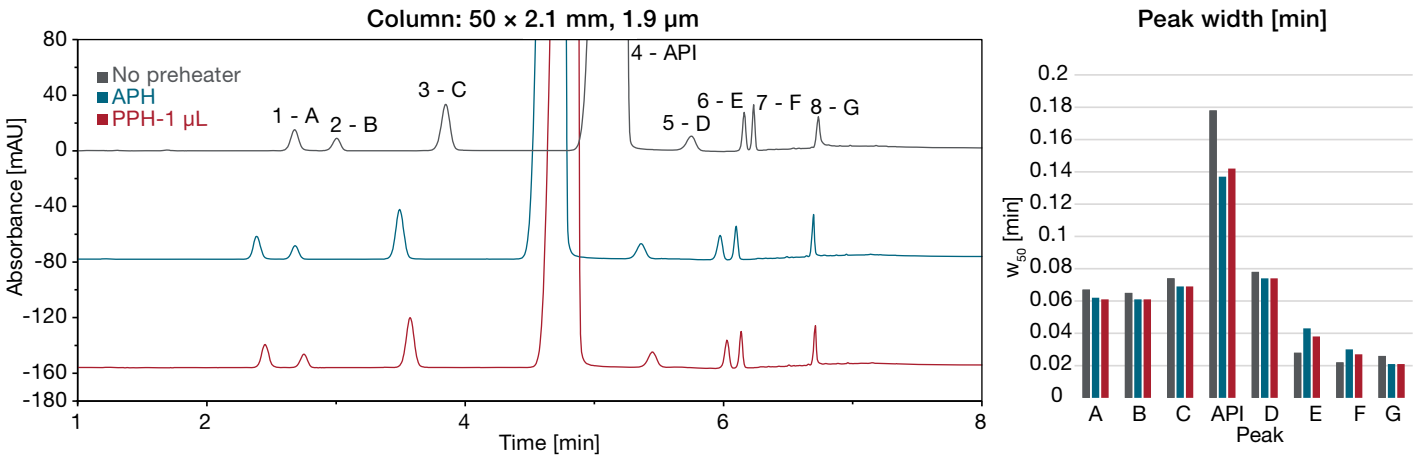

Figure 8. Separation results of mebendazole (API) and impurities (A-G) according to Ph. Eur monograph[5] method modified for UHPLC speed-up without mobile phase pre-heating (gray), with APH installed (teal), and with PPH-1 µL installed (red). Stationary phase: Thermo Scientific™ Hypersil GOLD™ Column, 50 × 2.1 mm, 1.9 µm (Cat. No. 25002-052130). Mobile phase A: 7.5 g/L ammonium acetate in water; mobile phase B: ACN. Flow rate: 0.395 mL/min. Column temperature: 40 °C (still air). Gradient: 0 min 20% B – 4.75 min 30% B – 6.33 min 90% B – 7.92 min 90% B – 7.95 min 20% B – 11.0 min 20% B.

The modified method was run with a smaller diameter column at a lower flow rate and under still air conditions. Again, APH and PPH-1 µL gave almost equivalent chromatographic results, with only slightly increased retention times for the PPH-1 µL, while without pre-heating, peak elution was clearly delayed because of the lower average column temperature. However, peak widths at half height (w_{50}) were less impacted compared to the original method, which is in response to the still air heating mode that mostly prevents the formation of a radial temperature gradient across the column cross-section. In fact, even lower peak widths were detected for impurities E and F without pre-heating than with pre-heating, which can be attributed to a secondary effect on the chromatography. The elution of these two peaks was so severely delayed that they were still on column when the steeper part of the gradient arrived. Thus, impurities E and F eluted as narrower peaks but with significantly reduced resolution.

From both applications, it is apparent that proper mobile phase pre-heating plays a critical role for the chromatographic outcome, even at moderate column temperatures. It not only supports adequate elution, peak shapes, and efficiency of a single run, but also day-to-day and site-to-site reproducibility when environmental conditions may vary. While in a certain range of conditions active and passive pre-heating can yield comparable outcomes, active pre-heating provides more reliable performance within the specification limits.

Conclusion

- Thermal mismatch of column and mobile phase causes retention shifts, peak distortion, and loss in efficiency. Mobile phase pre-heating improves chromatographic outcomes, reproducibility, and transferability.
- Under most conditions, active pre-heating obtains consistent and accurate eluent thermostatting, even across varying eluent compositions.
- Up to 1 mL/min, the heating performance of passive devices is only slightly inferior to active ones but drops significantly with higher flow rates.
- PPHs are beneficial for mobile phase cooling and outside the APH specification limits at high-flow/high-temperature conditions.
- Increasing PPH volume moderately improves pre-heating at flow rates above 1 mL/min.
- Significantly higher extra-column volume of PPHs can be beneficial to mitigate strong solvent effects but inherently increases peak dispersion, especially with small diameter columns.
- Organic solvent content of eluent can improve the pre-heating efficiency, which might be relevant in gradient elution.

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