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The Heat is On – Thermal optimization of HPLC systems with the KNAUER Eluent Preheating Cartridge

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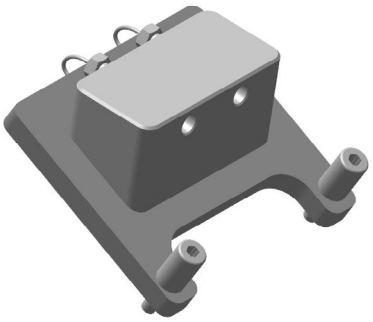

SUMMARY

Column temperature is a key parameter in HPLC method development. A stable temperature ensures repeatable measurements and can in many cases improve peak shape. The use of a column thermostat is considered standard in a modern HPLC system. A factor often neglected though is eluent temperature as it usually enters the system at ambient temperature. When both column temperature and flow rate are high, the relatively cold eluent will cool down the column internally and create a temperature gradient. In those cases, the KNAUER Eluent Preheating Cartridge stabilizes the eluent temperature before entering the column.

INTRODUCTION

In HPLC method development temperature control is a factor that can decide between a fast and efficient separation or a poorly resolved chromatogram. It is in general not possible to reliably predict a direct correlation between a raised temperature and chromatographic performance increase. Though, many HPLC application use cases show positive improvements with a raised temperature. Oftentimes, narrower peaks with a better gaussian shape can be observed.

Another benefit of elevated column temperature can be the reduced viscosity of the eluent. It can positively improve the column efficiency since flow velocity and therefore the exchange of substances is increased. Thus, shorter analysis times and decreased system pressures are achievable. As a result, many UHPLC applications require a raised temperature to enable ultrashort runtimes. In most common reversed phased applications, the column temperature is situated above 30 °C.

It is important to keep in mind that all chromatographic equilibriums are temperature dependent. Therefore, this principle also applies for LC-modes besides reversed phase such as ion-exchange or normal phase applications. A fact that is often overlooked is the influence of eluent temperature. In most cases the eluent supply is stored in bottles on top of the HPLC system at ambient temperature. Therefore, the solvents are at best at a stable temperature provided that the laboratory environment is air conditioned. In any case when working with an elevated column temperature a significant difference to the eluent bottle temperature is unavoidable.

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When relatively cold eluent is pumped into the column within the thermostat, it dwells only a short time before entering the column. The short distance of the column inlet capillary has a minimal surface area, so that heat flow by convection is inefficient.
As a result, the eluent cools the column internally creating an unwanted temperature gradient. This can lead to poor chromatographic performance and repeatability. As a rule of thumb, for flow rates greater than 0.5 ml/min, this effect is significant and should be carefully considered.
It is therefore crucial to normalize the eluent temperature before it enters the column. The KNAUER Eluent Preheating Cartridges available for the AZURA® CT 2.1 column thermostat are best suited for this task. They are combining minimal dead volume with maximum heat exchange capabilities. The Eluent Preheating Cartridges can be retrofitted to any CT 2.1 column thermostat within minutes.
The forced hot air column thermostat will heat up the heat exchanger within the cartridge to the target temperature. The eluent is now more efficiently tempered using heat conduction instead of heat convection. Eluent temperature is raised to target and is now entering the column with only a small difference.
In this TechNote the effect of the KNAUER Eluent Preheating Cartridge is showcased using a well-established USP application for the pharmaceutical quality control of citalopram.

SAMPLE PREPARATION

A standard solution with 6.25 µg/ml citalopram hydrobromide (Sigma Aldrich, product code 1002230688) was prepared with 50:50 methanol/water (v/v) as diluent. The buffer used in the mobile phase was prepared by dissolving 1 g of sodium acetate in 800 ml of water and adding 6 ml of triethylamine. The solution was adjusted with acetic acid to a pH of 4.6, and diluted with water to 1 l.
The eluent was prepared by mixing 80:20 acetonitrile/buffer and adjusting the pH to 5.0.

RESULTS

The citalopram hydrobromide was determined using a method according to a monograph in the USP 44–NF 39 (2021)[1,2]. The column temperature was set to 50 °C and an injection volume of 20 µl was used. A flow rate of 1 ml/min was set and the method run time was 45 min. After the initial measurements the eluent preheating cartridge was integrated into the flow path and the citalopram hydrobromide standard was measured again **(Fig. 1)**.

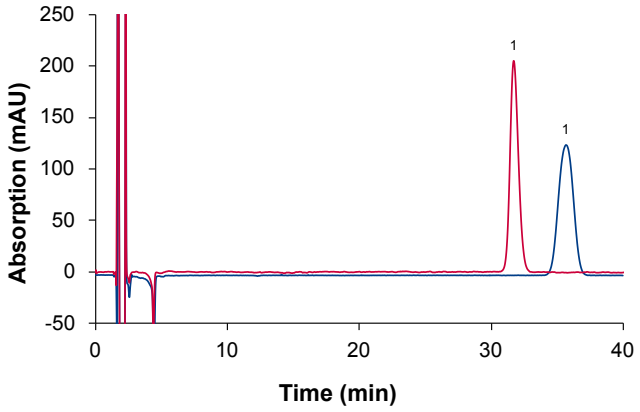

Fig. 1 Comparison chromatogram of USP-Method; (1) Citalopram hydrobromide A; (blue) Eluent Preheating Cartridge not installed; (red) Eluent Preheating Cartridge installed; 20 µL injection volume; temperature 50 °C; flow rate 1 ml/min; Eurospher II 100-5 C8 150 x 4.6 mm

To show the effect of eluent tempering in a more modernized method, the original USP method was scaled down to a shorter column with a smaller inner diameter and a smaller particle size. For calculating the scale-down the KNAUER HPLC Method Converter software was used, ensuring that the deviation is acceptable according to USP621 (column length / particle size = -25 to 50%) (Free download **KNAUER HPLC Method Converter**). With the new calculated parameters an injection volume of 2.5 µl was chosen, together with a flow rate of 0.7 ml/min. The column temperature was kept at 50 °C and the measurements were repeated with and without the eluent preheating cartridge in the flow path **(Fig. 2)**.

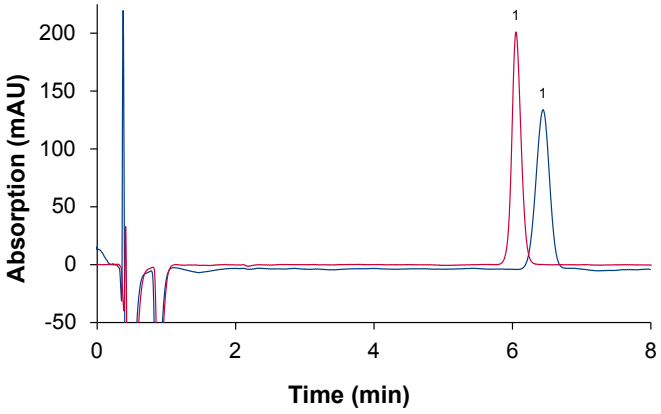

Fig. 2 Comparison chromatogram of scaled-down USP-method; (1) Citalopram hydrobromide A; (blue) Eluent Preheating Cartridge not installed; (red) Eluent Preheating Cartridge installed; 2.5 µL injection volume; temperature 50 °C; 0.7 ml/min; Eurospher II 100-3 C8 100 x 2 mm

A drastic effect was observed on both methods when comparing measurements performed with and without eluent preheating cartridge **(Fig. 1, Fig. 2)**. The citalopram hydrobromide elutes earlier and sensitivity is improved as a result of increased peak height and improved peak shape **(Fig. 3)**. The signal to noise ratio was improved by a factor of 10.

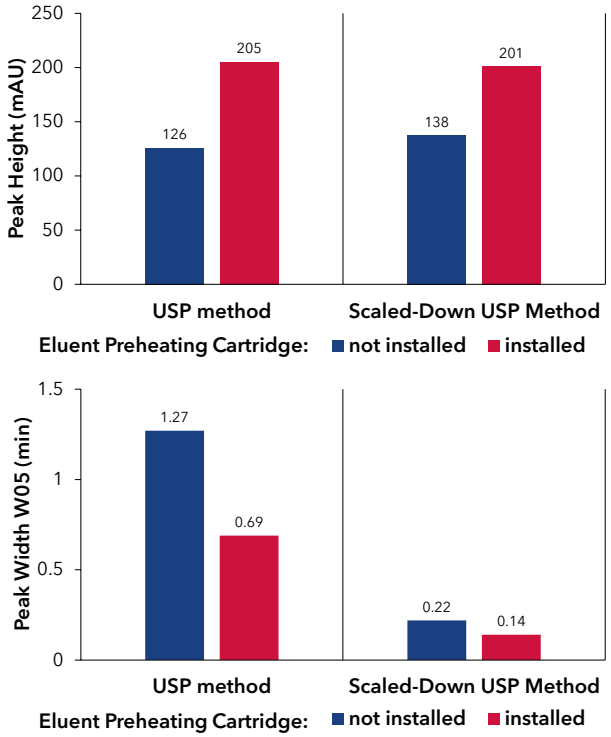

Fig. 3 Improvement of chromatographic parameters when using the eluent preheating cartridge

Observation of the column thermostat with an infrared camera has shown that the capillary connected to the outlet of the eluent preheating cartridge has a more equalized temperature than the one entering the column directly **(Fig. 4)**.

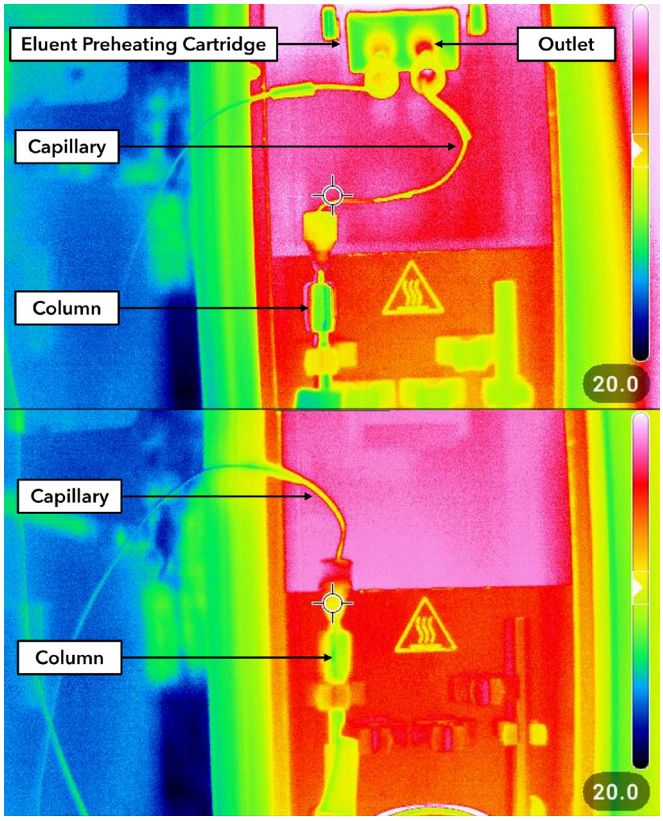

Fig. 4 Infrared camera pictures of column thermostat with (top) and without eluent preheating cartridge (bottom)

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7. CONCLUSION

Whenever working with tempered columns, eluent temperature stabilization is key. If the target temperature differs from the room temperature by a large amount an eluent preheating cartridge should be considered. The eluent preheating cartridges available for the AZURA CT 2.1 column thermostat come in two different volume sizes for minimal overall dead volume. The internal diameter of the installed cartridge can be selected to suit the system configuration: 0.18 mm ID for HPLC and 0.1 mm ID for UHPLC and ULDC applications. They can be retrofitted to any CT 2.1 with minimal effort by the user. Since chromatographic performance may drastically increase, eluent tempering can in some cases make the difference between reaching LOD and failing system suitability. Therefore, the use of the AZURA Eluent Preheating Cartridge is highly recommended for all applications at raised column temperatures and flow rates greater than 0.5 ml/min.

MATERIAL AND METHODS

Tab. 1 Method settings

Parameter	Settings	
	USP method	**Scaled-down method**
Mode	Isocratic	
Runtime	45 min	8 min
Flowrate	1 ml/min	0.7 ml/min
Mobile Phase	20:80 Acetonitrile/TEA Buffer pH5.0 (v/v)	
Injection volume	20 µl	2.5 µl
Column	Eurospher II 100-5 C8 150 x 4.6 mm	Eurospher II 100-3 C8 100 x 2 mm
Column temperature	50 °C	
Detection	UV	
Wavelength	239 nm	
Data rate	10 Hz	20 Hz

Tab. 2 System configuration

Instrument	Description	Article No.
System	AZURA® Analytical (U)HPLC 862 bar System	AZURA862
Pump	AZURA® P 6.1L High Pressure Pump with 5 ml pump head, stainless steel	APH35GA
Injection	AZURA® Autosampler AS 6.1L with 100 µl loop	AAA10AA
Column tempering	AZURA® CT 2.1	ATC00
Column tempering	Eluent Preheating Cartridge for CT 2.1	A05853
Column	Eurospher II 100-5 C8 150 x 4.6 mm	15EE081E2J
Column	Eurospher II 100-3 C8 100 x 2 mm	10BE081E2G
Detector	AZURA® DAD 6.1L	ADC11
Flow cell	High Sensitivity KNAUER LightGuide UV Flow Cell Cartridge	AMD59XA
Capillaries	AZURA® Analytical MarvelXACT™ StartUp kit for ULDC & UHPLC systems, Set of capillaries, connectors and adapters	AZF110
Software	ClarityChrom® 9.1.0 - Workstation, autosampler control included	A1670
Software	ClarityChrom® 9.1.0 - PDA extension	A1676

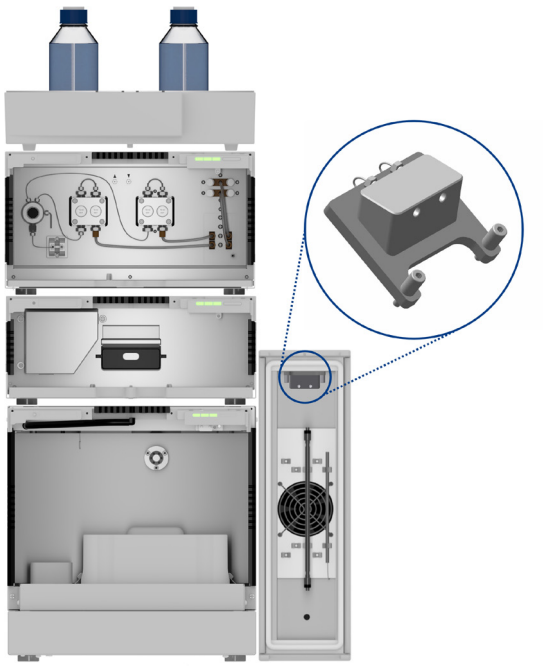

Fig. 5 AZURA Analytical system setup; from top to bottom: eluent tray with bottles, pump, diode array detector (PDA), autosampler; right: thermostat with columns and eluent preheating cartridge.

REFERENCES

1] United States Pharmacopeial Convention Citalopram Hydrobromide, 44th Edition., United States Pharmacopeial Convention, Rockville, MD, 2021.

[2] **KNAUER HPLC Method Converter** 2023.