

Application Note

## The Importance of Column Compartment Thermostating and Preheating for Temperature Sensitive Separations in Liquid Chromatography

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## Abstract

Understanding the mechanism and impact of column compartment thermostating and mobile phase preheating on chromatographic separations is important in liquid chromatography. This application note demonstrates the effect of differences in column compartment design on resolution and selectivity of a critical pair of temperature sensitive compounds across various LC instrument vendors. General consideration of column compartment thermostating design and mobile phase preheating during method development and method transfer is also provided.

### Benefits

- Expanded choice of column compartments enhances ACQUITY Arc and ACQUITY Arc Bio Systems as an ideal platform for routine analysis and method development
- ACQUITY Arc CMA provides active preheating and extended column switching capability useful for method transfer and method development

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## Introduction

Separation temperature plays an important role in high performance liquid chromatography (HPLC) since it can influence retention time, selectivity, and peak shape. While temperature is one of the key factors to control during development and transfer of LC methods, the understanding of column thermostating and mobile phase preheating and their impact on chromatography are often incomplete.

In today's analytical instrument market, most LC systems include either a dedicated column compartment or options of various types of column compartments. In general, there are two common types of column compartments, block heater and circulation air types.<sup>1,2</sup> The different thermostating mechanisms can result in differences in heat transfer efficiency, temperature equilibrium, and the creation of a temperature gradient in the column. For adequate mobile phase preheating, most LC vendors provide preheaters that will heat the mobile phase to the set method temperature prior to solvent introduction into the column. Mobile phase preheating can be passive or active. Passive preheaters are capillaries of a specified length that are in direct solid contact to a temperature-controlled surface in the column compartment. Active preheaters employ an internal heating element to actively control the eluent temperature. All these variables can impact the

transferability of temperature sensitive separations.

To illustrate this, we conducted a temperature sensitive separation on the ACQUITY Arc System configured with the ACQUITY Column Manager (CMA), a new extension of column compartments to ACQUITY Arc and ACQUITY Arc Bio family.<sup>3</sup> The same experiments were also carried out on LC systems from various vendors with different column thermostating and preheater designs. While many instrument parameters can have impact on LC separations, this application note will focus on the impact of column compartment characteristics and mobile phase preheating.

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## Experimental

### Sample Description

Waters Analgesic Mix Standard (p/n 186006350 <<https://www.waters.com/nextgen/us/en/shop/standards-reagents/186006350-analgesic-mix-standard.html>> ) was prepared in 90% water/10% acetonitrile at a concentration 50 µg/mL.

### Method Conditions

#### LC Conditions

LC system:	ACQUITY Arc with CMA
Column temp.:	35 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C
Equilibrium time at each temp.:	30 min
Active preheater:	with preheating – preheater enabled without preheating – preheater disabled
Detection:	2998 Photodiode Array (PDA) Detector
Wavelength:	245 nm

## LC Conditions

Column:	XBridge BEH C <sub>18</sub> Column, 130 Å, 3.5 µm, 4.6 mm x 150 mm (p/n 186003034)
Injection volume:	5 µL
Flow rate:	1 mL/min
Mobile phase A:	Water/0.1% formic acid
Mobile phase B:	Acetonitrile/0.1% formic acid
Gradient:	15 to 30% B/7 min

## Data Management

Chromatography software:	Empower FR3
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## Results and Discussion

As previously studied, separation of an analgesics mix is temperature sensitive.<sup>4</sup> Figure 1 compares the analgesics mix separation at set column compartment temperatures with the active preheater enabled or disabled on ACQUITY Arc System configured with CMA. In the sample mix, component 6 (phenactein), and 7 (salicylic acid) are a critical pair with resolution and selectivity being strongly impacted by temperature. This pair was used as a probe to study the impact of column compartment thermostating and preheating on LC separations.

### Impact of Preheating

As shown in Figure 1, a dramatic difference in selectivity and resolution of the critical pair was observed with and without preheating of the mobile phase (column compartment was set to the temperature shown in Figure 1). The general trend is that the separation profile at a higher temperature without preheating (see

Figure 1B at 45 °C) was similar to the separation profile at a lower temperature with preheating (see Figure 1A at 35 °C). Without preheating, the mobile phase entering the column has not fully equilibrated with the set temperature of the column oven, therefore the separation is carried out at a lower temperature than the set temperature of the column compartment. Even though all of analgesics mix peaks shift to shorter retention times with increasing temperature, the degree of the retention time shift with preheating was greater than the retention time shift without preheating.

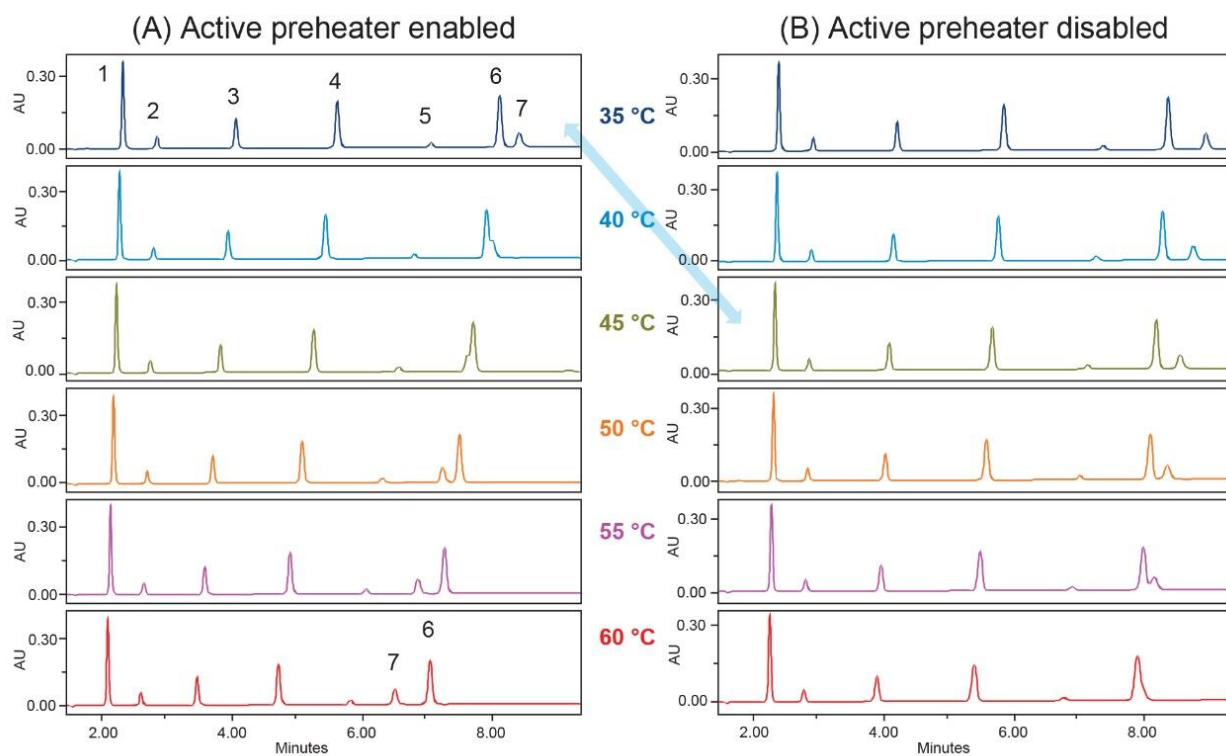


Figure 1. Temperature impact on analgesics mix separation acquired on an ACQUITY Arc System with CMA. (A) with active preheater enabled (with preheating); (B) with active preheater disabled (without preheating).

Figure 2 shows the retention time of peak 6 changing with temperature with the active preheater enabled (with preheating) and with active preheater disabled (without preheating). With the preheater enabled, the slope of retention time with temperature is greater than the slope without preheating. This indicates that the mobile phase in the analytical column aligned with the set temperature more rapidly with preheating than without preheating. With the preheater disabled, there might be a mismatch of the temperature of the column wall and mobile phase flow within the column, creating a temperature gradient across the column.

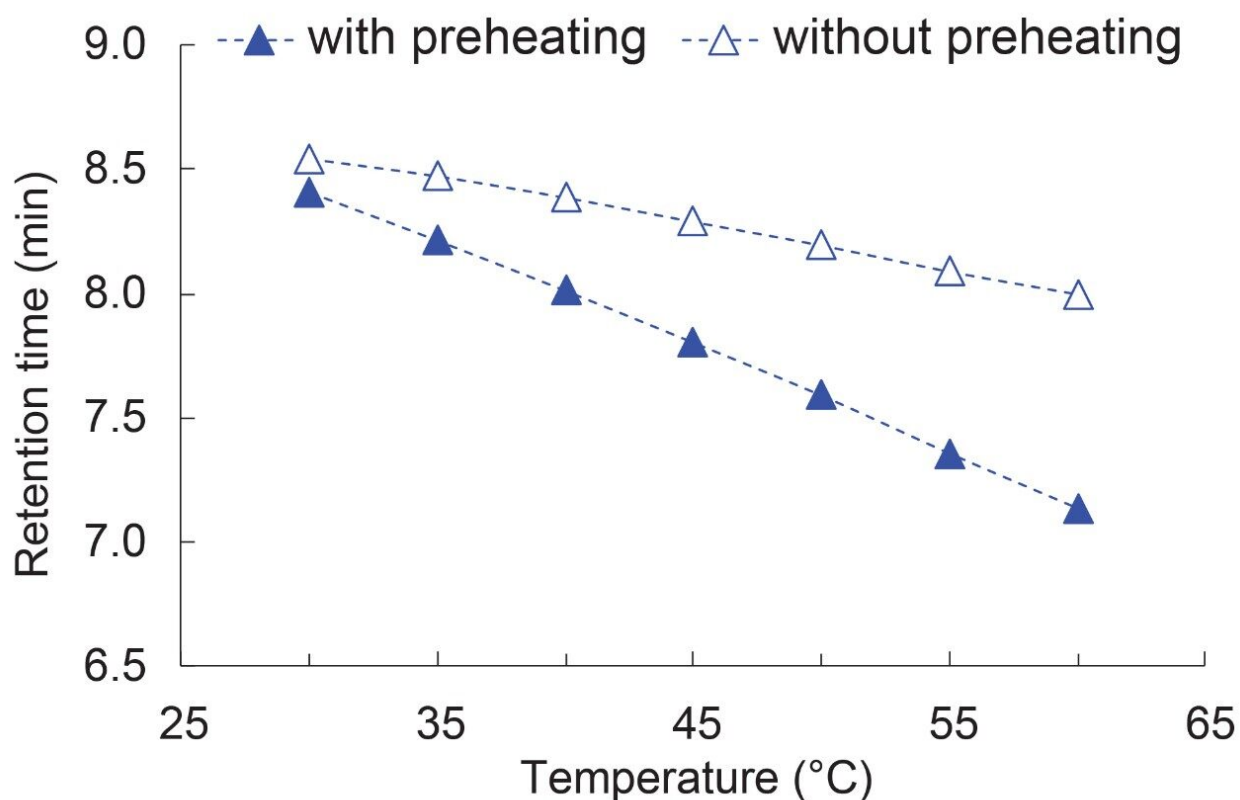


Figure 2. Change of retention time of peak 6 with temperature under active preheater enabled (with preheating) and disabled (without preheating) on an ACQUITY Arc System with CMA.

### Impact of Column Compartment Thermostatting

In addition to solvent preheating, column thermostatting design can impact the separation as well. Four UHPLC systems with different types of column compartment thermostatting mechanisms were selected for the study (Table 1). Among them, the ACQUITY Arc System (with active preheater) and vendor X (with passive preheater) are block heater type. Vendor Y (with passive preheater) and vendor Z (with no preheater) are air circulation type. In general, the block heater type provides efficient real-time column wall heating since the column directly contacts the heat source. Air circulation type is based on air bath concept. Compared to metal, air is a poor heat conductor, hence an air bath is less effective at controlling the column temperature in real-time. However, the effectiveness of column heating can be enforced by circulating the air in the air circulation type column compartment.<sup>2</sup>

LC systems	Column compartment type		Preheating
	Block heater	Air circulation	
ACQUITY Arc	√		Active
Vendor X	√		Passive
Vendor Y		√	Passive
Vendor Z		√	<b>No</b>

Table 1. List of column compartment types and preheating features of the four UHPLC systems.

For the ACQUITY Arc System and vendor X, which both have similar U-shape design block heaters, similar selectivity, and resolution of the critical pair was obtained with preheating (Figure 3A) and without preheating (Figure 3B) respectively. The preheater types (active vs. passive) do not have significant impact on the separation profile of the critical pair.

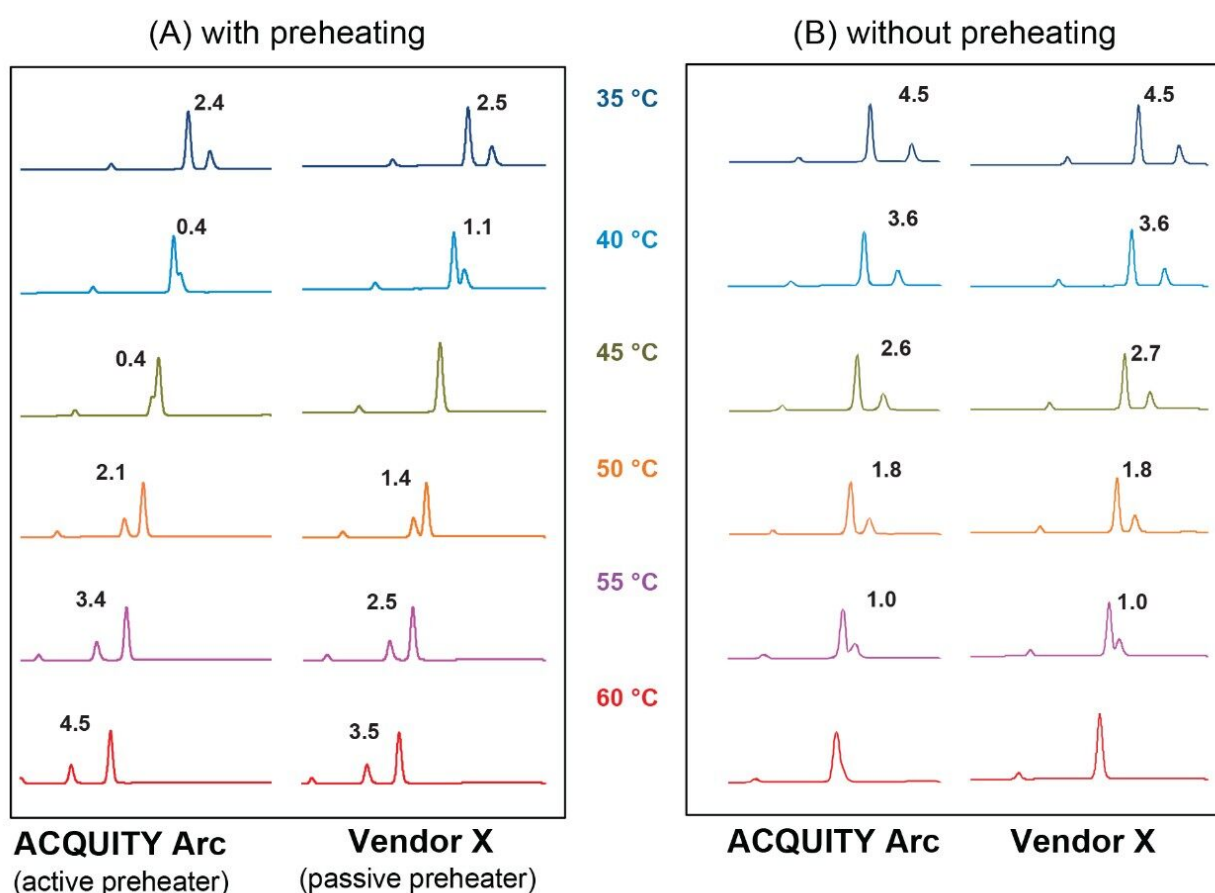


Figure 3. Separation profile (resolution value was labeled) of the critical pair in analgesics mix at elevated temperatures for block heater types: ACQUITY Arc and vendor X.

(A) with preheating, for ACQUITY Arc, the active preheater was enabled. For vendor X, passive preheater was in the flow path.

(B) without preheating. For ACQUITY Arc, the active preheater was disabled. For vendor X, passive preheater was taken out of the flow path.

On the other hand, without preheating mobile phase, the selectivity and resolution of the critical pair on the block heater type (Figure 4A, ACQUITY Arc and vendor X) is significantly different from those on air circulation type (Figure 4B, vendor Y and vendor Z). On block heater type, the separation profile of the critical pair at higher temperature (see Figure 4A, 45 °C) is similar to the separation at lower temperature on air circulation type (see Figure 4B, 35 °C).



(A) Block heater type

(B) Air circulation type

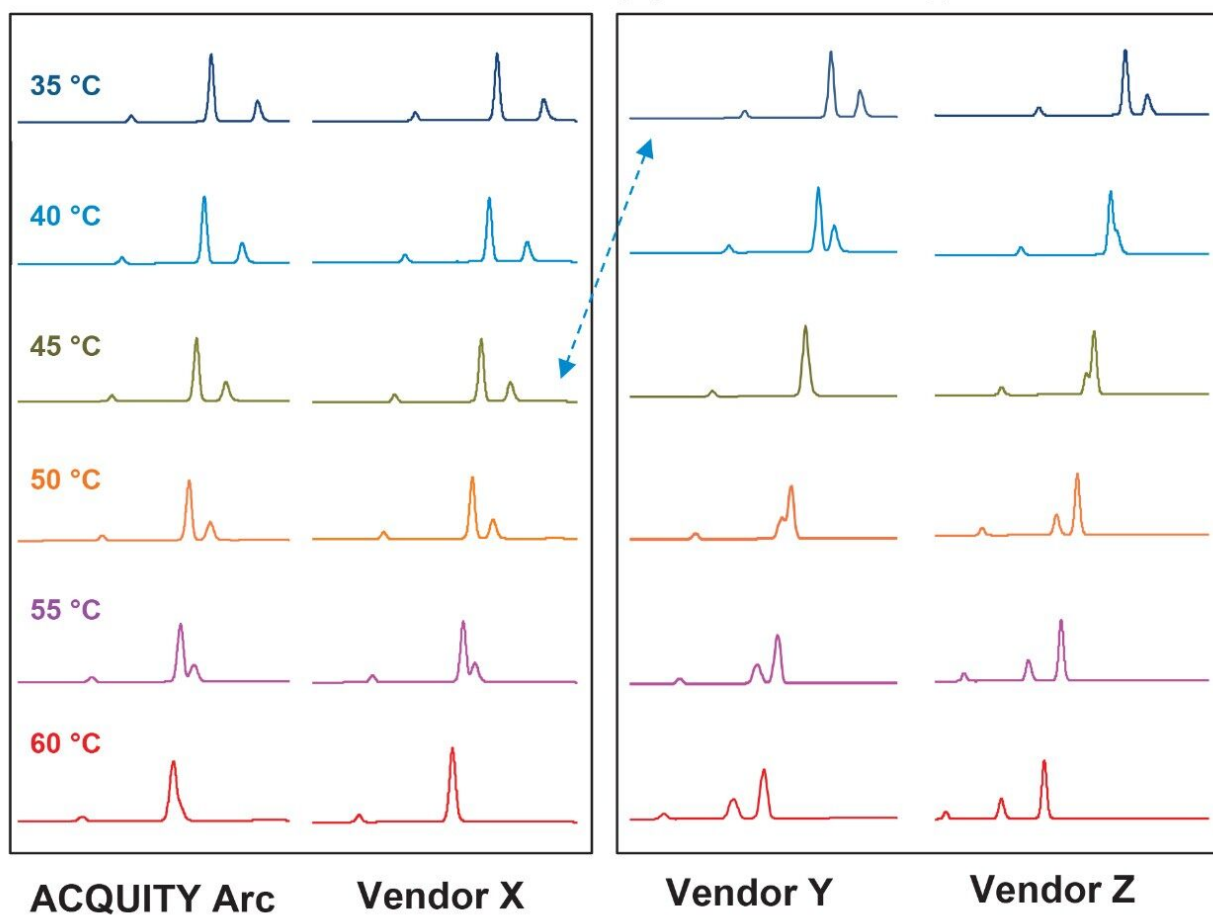
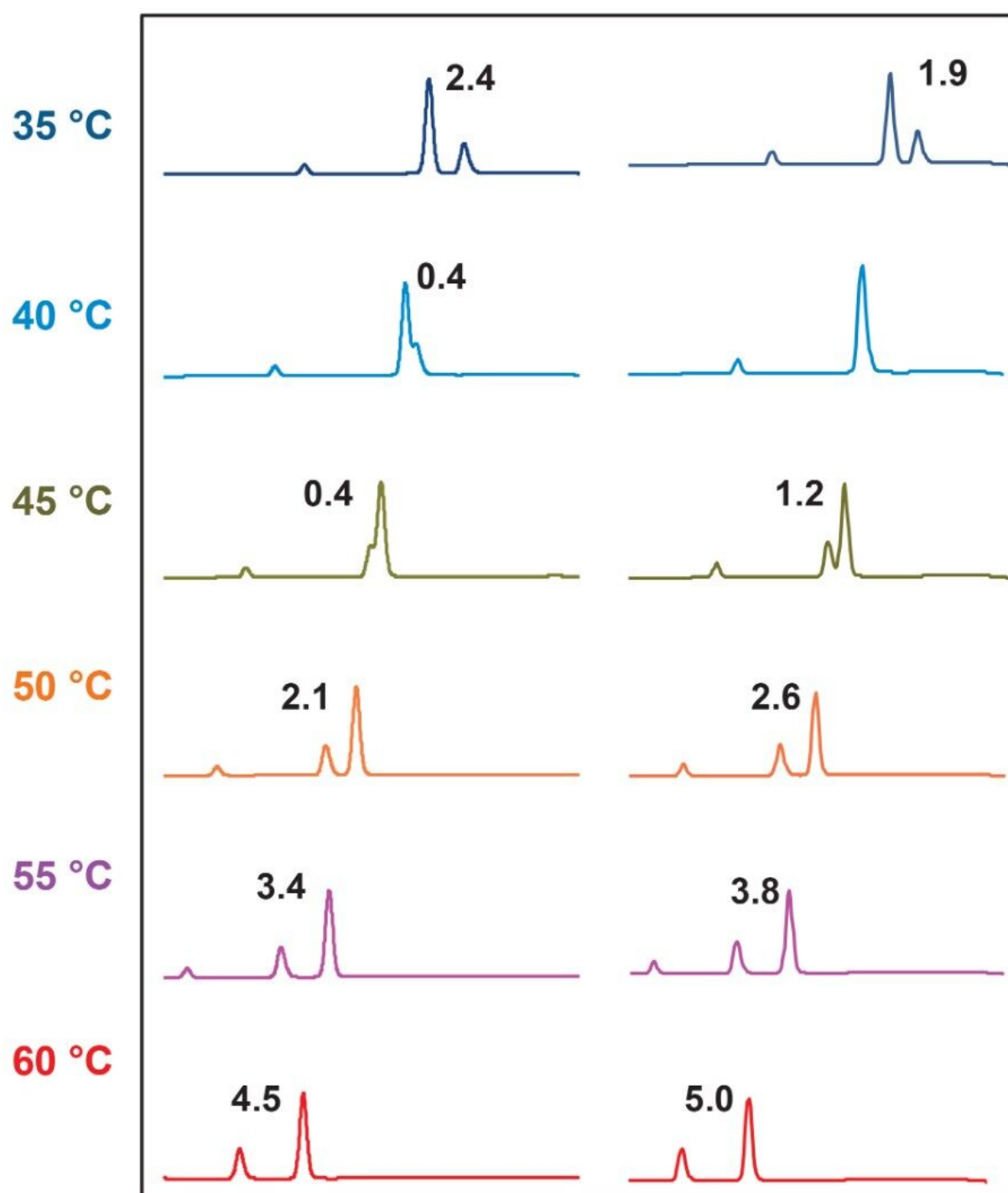


Figure 4. Separation profile of the critical pair in analgesics mix at elevated temperatures without preheating the mobile phase on (A) block heater type, ACQUITY Arc and vendor X and (B) air circulation type, vendor Y and Z system.

However, when preheating the mobile phase, the separation profile of the critical pair behaved similarly on block heater type (ACQUITY Arc System) and air circulation type (vendor Y)(Figure 5). This observation indicates that the difference on the temperature equilibrium within the column due to different thermostating designs can be minimized by preheating the mobile phase.

Block heater

Air circulation



**ACQUITY Arc**  
(active preheater)

**Vendor Y**  
(passive preheater)

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Figure 5. Separation profile (resolution is labeled) of the critical pair in analgesics mix with preheating at elevated temperatures on block heater type, ACQUITY Arc and air circulation type, vendor Y.

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## Conclusion

In this application note, we carried a temperature sensitive separation study on ACQUITY Arc System configured with CMA and LC systems with different thermostating designs from various vendors. The study demonstrated that the column compartment characteristics, including thermostating type and mobile phase preheating, greatly impact temperature sensitive separations. Some general considerations when developing and/or transferring methods among different LC systems are:

- When developing a new method, always use a preheater
- If the existing method has been developed without a preheater, it is recommended to use the same type of column compartment thermostating

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## References

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ACQUITY Arc System <<https://www.waters.com/134844390>>

Empower Chromatography Data System <<https://www.waters.com/10190669>>

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