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응용 자료

Method Transfer from Agilent 1100 Series LC System to the ACQUITY UPLC H-Class System: The Effect of Temperature

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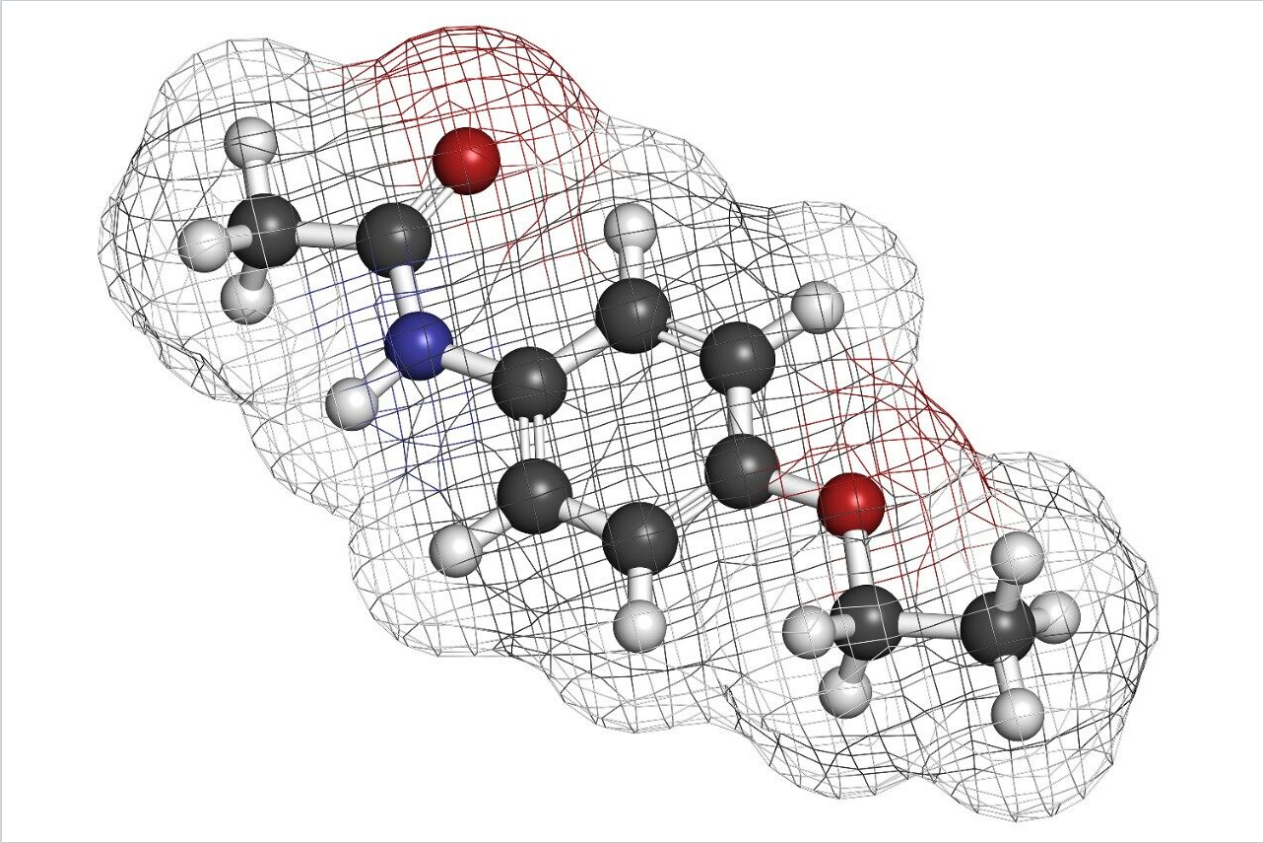


This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the comparability of temperature selectivity effects on HPLC method transfer from an Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System.

Benefits

Methods transfer from an Agilent 1100 Series LC System to a Waters ACQUITY UPLC H-Class System with a CH-A can be successful over a range of temperatures.

Introduction

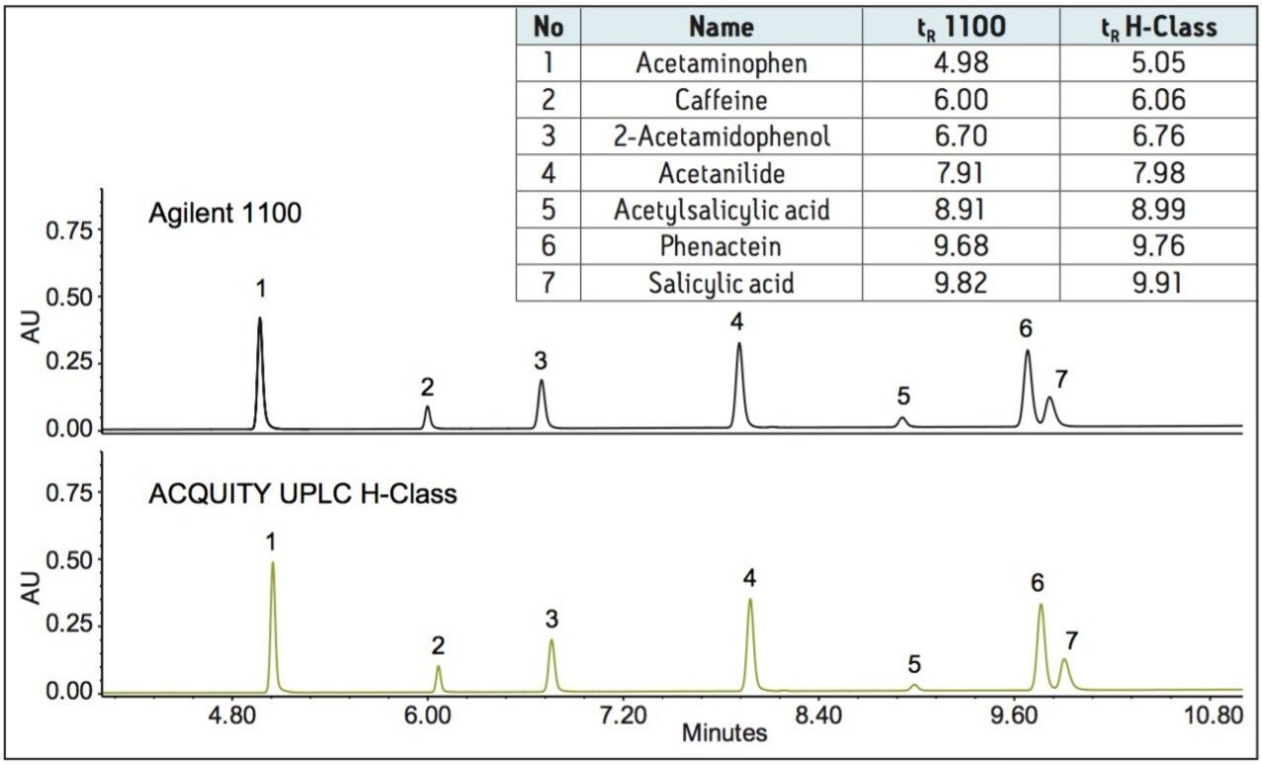
Given the high investment in instrumentation, analytical laboratories often have the need or desire to transfer methods across available systems, regardless of whether those systems come from a single or from multiple instrument manufacturers. For methods that are temperature-sensitive, transferring legacy HPLC methods across systems from different manufacturers can be challenging. Instrument modes for heat transfer can vary: instrument attributes can include active or passive preheating of the mobile phase and/or static or convection heating and cooling of the column. The differences in heat transfer approaches can affect the fidelity of the separation. If the separation is not preserved, the simplest approach to resolving poor method transfer is to adjust the set column temperature.[1] If this option is not permitted, the method may need to be redeveloped, or deemed untransferable, both resulting in added costs from the loss of instrument time and the loss of the analytical chemist's time.

Results and Discussion

Method transfer from the Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System with a single column compartment (CH-A) was performed. To account for differences across the systems, each instrument's dwell volume was measured[2] and, for the ACQUITY UPLC H-Class System, the appropriate gradient delay was entered using gradient SmartStart Technology.[3] To evaluate the effect of temperature control, each system was tested both with and without mobile phase pre-heating. The Agilent 1100 Series LC

System included a passive pre-heater (3 µL) and the ACQUITY UPLC H-Class System contained an active pre-heater. For analyses without pre-heating, the Agilent 1100 Series LC System was plumbed to bypass the passive pre-heater (directly from the injector valve to the column inlet), and the ACQUITY UPLC H-Class System's active pre-heater was set to active, disabled mode in the instrument console (i.e., no active pre-heating).

On an Agilent 1100 Series LC System, an HPLC separation of analgesics was found to produce selectivity differences over a temperature range of 36 °C to 60 °C. Given the impact of temperature on the separation, method transfer to the ACQUITY UPLC H-Class System was also evaluated over the same temperature range as shown by a representative chromatogram at 36 °C (Figure 1). The results showed comparable retention times and similar retention time trends on both systems over the temperature range (Figure 2, red and blue lines). Further confirmation of the equivalency was provided by the retention time behavior of phenacetin and salicylic acid (Figure 3). The same selectivity changes were observed at temperature intervals of 2 °C, confirming the high precision across the two systems.



No	Name	t_R 1100	t_R H-Class
1	Acetaminophen	4.98	5.05
2	Caffeine	6.00	6.06
3	2-Acetamidophenol	6.70	6.76
4	Acetanilide	7.91	7.98
5	Acetylsalicylic acid	8.91	8.99
6	Phenactein	9.68	9.76
7	Salicylic acid	9.82	9.91

Figure 1. Separation of an analgesic mix on an Agilent 1100 Series LC System and an ACQUITY UPLC H-Class System at 36 °C. Comparable retention times were observed on both instruments.

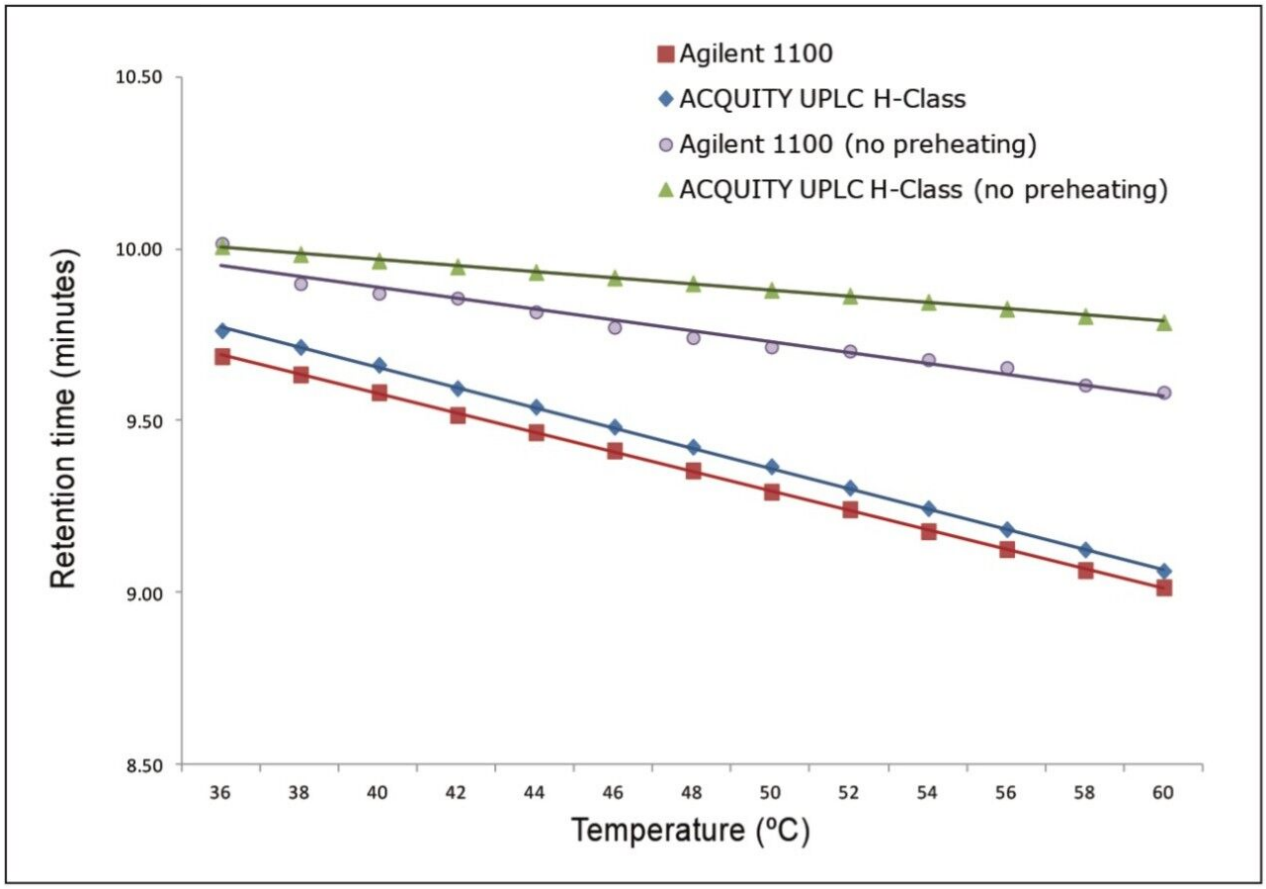


Figure 2. The effect of column temperature control on retention time for phenacetin. Both the ACQUITY UPLC H-Class System and the Agilent 1100 Series LC System exhibited a reduction in retention time with increasing temperature. For each system, mobile phase pre-heating resulted in greater change in retention time as a function of temperature.

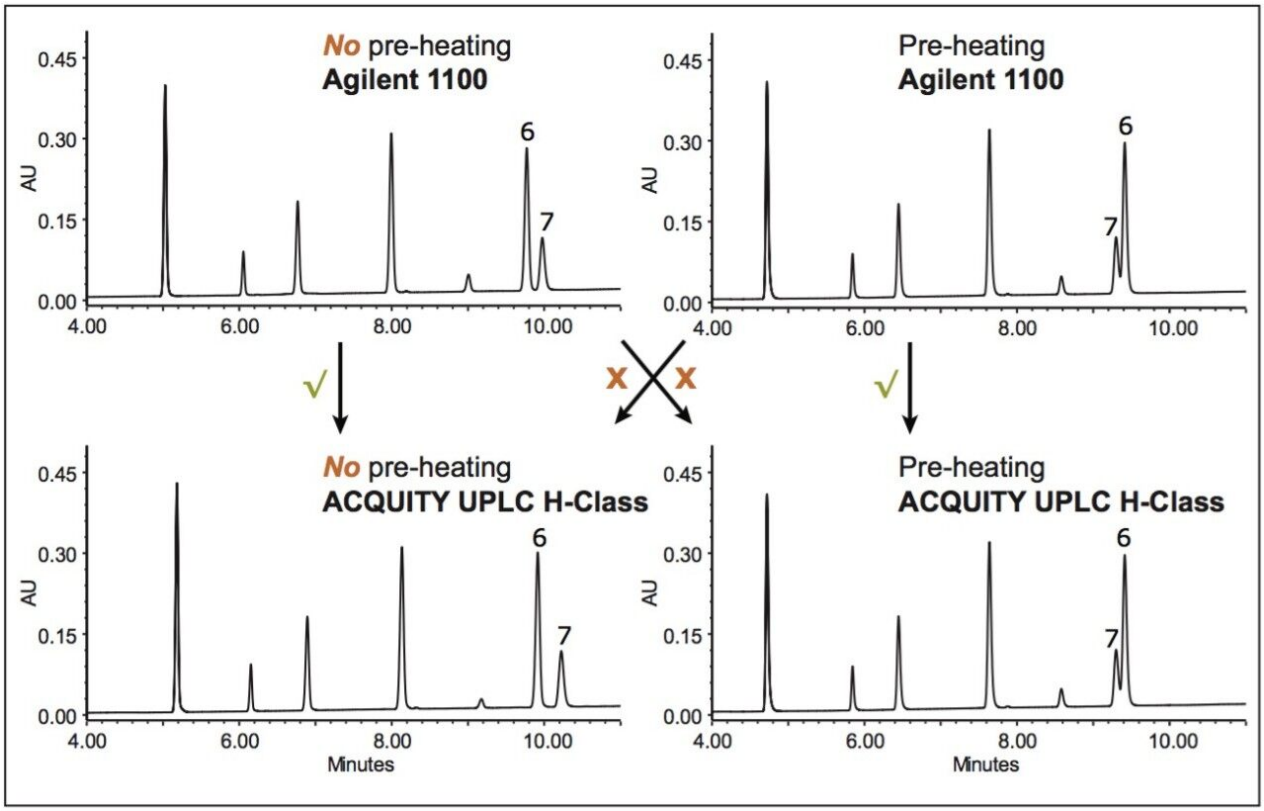


Figure 3. The effect of temperature on the separation of an analgesic mix. Both the Agilent 1100 Series LC System and the ACQUITY UPLC H-Class System showed similar selectivity changes with temperature for phenacetin (6) and salicylic acid (7).

While mobile phase pre-heating at higher temperatures can improve column performance by reducing axial and radial temperature gradients,[4] not every legacy HPLC method may use this type of temperature control. Therefore, the same experiments were repeated on both systems without mobile phase pre-heating. Under these conditions, comparable retention times were observed on both the Agilent 1100 Series LC System and the ACQUITY UPLC H-Class System (Figure 4). However, over the entire temperature range (36 °C to 60 °C), both sets of analyses produced minimal changes in retention time as a function of temperature (Figure 2, green and purple lines). In addition, without mobile phase pre-heating, no selectivity changes were observed above 42 °C as in the first set of experiments (i.e., those which used mobile phase pre-heating) (Figure 4). It is important to note: for this example, at temperatures above 42 °C, methods transfer – even on the same system – would be unsuccessful if the changes are made to mobile phase pre-heating. Thus, when transferring a method the use - or lack - of mobile-phase preheating should be considered.

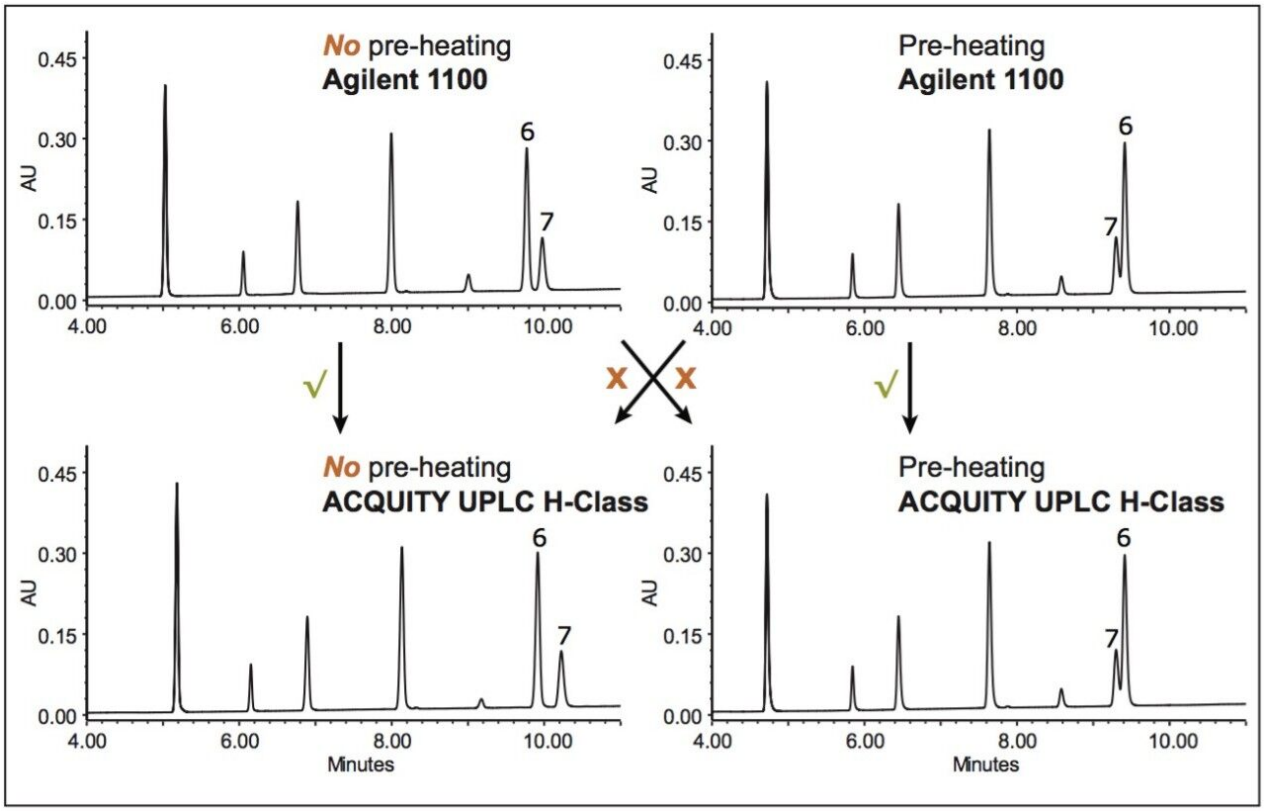


Figure 4. Effect of mobile phase pre-heating on an analgesic separation at 46 °C. With the same pre-heating configuration, the Agilent 1100 Series LC System and the ACQUITY UPLC H-Class System produced comparable retentivity (√). However, selectivity differences for phenacetin and salicylic acid were observed between those separations run with mobile phase-preheating and those run without (X).

Conclusion

Temperature control in an HPLC method can have a significant impact on the success of methods transfer. In this discussion, an HPLC method was successfully transferred from an Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System with a single column compartment. The effect of temperature upon the separations was found to be comparable. However, each system produced different results depending on whether or not mobile phase pre-heating was used. For this set of experiments, temperature selectivity effects were only observed using mobile phase pre-heating. Thus, the impact of temperature control should be considered in methods transfer. However, with or without mobile phase pre-heating, we have demonstrated successful method transfer from an Agilent 1100 Series LC System to an ACQUITY UPLC H-

Class System with a single column compartment (CH-A).

References

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