ZirChrom Columns and the Metalox® 200-C High Temperature Liquid Chromatography Column Heater - The Future of HPLC has Arrived.

Welcome to the fifth issue of ZirChrom's electronic newsletter. In response to the overwhelming interest regarding a previous issue on high temperature liquid chromatography, this newsletter will revisit temperature as a cost-saving HPLC tool and the practical application of temperature in HPLC using the Metalox® 200-C column heater.

ELEVATED TEMPERATURE - HOW IT WORKS

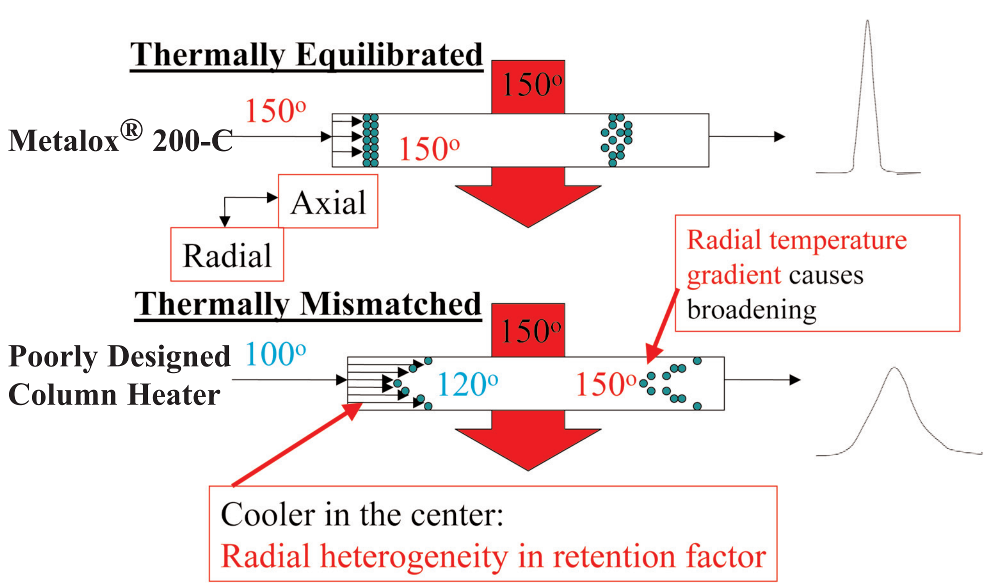
Chromatographers have long known that modest increases in operating temperature can dramatically improve both the efficiency and speed of an HPLC separation. For example, in a recent article, David V. McCalley found large increases in the efficiency for basic compounds at elevated temperature[1]. McCalley suggested both that basic compounds should be analyzed at higher temperature and that new columns should be developed that are stable at high temperature.

Using temperature to affect profound improvements in the speed of analysis, predicted by Antia and Horvath over a decade ago, can be realized through three main effects[2]. Firstly, the viscosity of the mobile phase is decreased, so that higher flow rates are possible with existing equipment without increasing backpressure. Secondly, at higher temperature, the diffusion rate of analytes increases so the losses in efficiency associated with increased flow rates are minimized[3]. Finally, at elevated temperature, the kinetics of the interactions between the analytes and the stationary phase are faster. This will lower the overall analysis time, and often reduce or eliminate peak tailing.

ELEVATED TEMPERATURE - CHALLENGES

Historically, there have been three main challenges to implementing high temperature liquid chromatography: (1) column thermal stability, (2) thermal mismatch broadening, and (3) analyte stability. Elevated temperature can be used with conventional HPLC columns, but can significantly reduce column lifetime. At 60 °C, most silica-based columns degrade very quickly, unless operating conditions and buffers are chosen very carefully. To get good column life, a column based on a thermally stable support such as zirconia should be used. Thermal mismatch band broadening is the distortion of chromatographic peaks caused by a radial inequality of temperature across the diameter of the column. This phenomenon is caused by poor heater design; by not ensuring that the incoming mobile phase temperature matches that of the column. The four sensor cascading-loop design of the Metalox® 200-C eliminates thermal mismatch band broadening by ensuring that the temperature within the interior of the column is equilibrated with the temperature of the exterior of the column (See Figure 1).

Figure 1. Thermal Mismatch Band Broadening.



Some chromatographers (particularly in the pharmaceutical industry) are concerned about the effect of these techniques on temperature-sensitive compounds. Dr. Jon Thompson and Dr. Peter Carr have clearly demonstrated that for high temperature LC, analytes must be thermally stable only for the time scale of the chromatographic run[4]. Their study of a number of pharmaceuticals shows that as the time an analyte is exposed to high temperature decreases (as the temperature increases, flow rate is increased, and dwell time is decreased), the likelihood and extent of an on-column dedgredation is greatly diminished.

ELEVATED TEMPERATURE VS. SHORTER COLUMNS FOR FAST LC

The case has been made by others that one may simply shorten the column to increase analysis speed. However, often neglected in these discussions is the loss of resolution that must occur if a shorter column is employed. The following compares and contrasts the benefits/drawbacks of using shorter columns versus elevated column temperature for fast liquid chromatography:

Table 1. Comparison of temperature versus shorter columns for fast liquid chromatography.

	Shorter Columns	**Elevated Temperature**
Flexibility	Not Flexible. A new column needs to be purchased each time you want to adjust speed.	Very Flexible, a wide range of temperatures allow you to adjust speed upon demand and fine tune your separation.
Column/Analyte Stability	The thermal stability of the column and analyte is not a concern.	You must have a column that is up to the challenge of high temperature LC. The gain in analysis speed often prevents any undesirable on-column reactions.
Resolution $R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1+k'}\right)$	Decreasing the column length directly translates into a loss in plate count, as N is directly proportional to L; this then translates into a loss in resolution according to the general resolution equation. This initial loss in resolution can be overcome somewhat by increasing the rentention factor through a weakening of the mobile phase, however this is counterproductive in terms of improving speed.	The use of elevated temperature to decrease the viscosity of the eluent allows higher flow rates and therefore faster analyses, **without sacrificing resolution** through a decrease in plate count.

ELEVATED TEMPERATURE - SAFETY CONCERNS

Some scientists worry that their mobile phase will boil in the columns at higher temperatures causing damage to their equipment and/or a safety hazard in the lab. It is important to remember, however, that the extremely high pressure in an HPLC column raises the boiling points of HPLC solvents, so that flashing cannot occur. Below 80 °C, it is not necessary to modify a standard HPLC system to operate safely. The tubing from the column to the detector and from the detector to the waste receptacle is sufficient to cool the mobile phase back to room temperature. Above 100 °C, adding a backpressure regulator, such as the one included with the Metalox® 200-C, after the detector to keep about 30 bar on the system is generally enough to prevent flashing. At very high temperature (~150 °C), it is important to cool the mobile phase downstream of the column and before the detector. The Metalox® 200-C incorporates this feature by using its efficient heated exchanger design to cool 200 °C water mobile phase at a flow rate of 6 ml/min down to 80 °C before leaving the column heater.

Another safety concern addressed by the engineers of the Metalox® 200-C is the possibility of a small leak in the column connection inside the heating chamber. The smaller, energy efficient, oven design of the Metalox® 200-C eliminates the large air volumes that can trap volatile vapors from a connection leak.

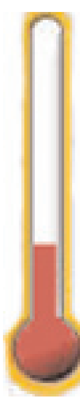
ELEVATED TEMPERATURE - THE BOTTOM LINE

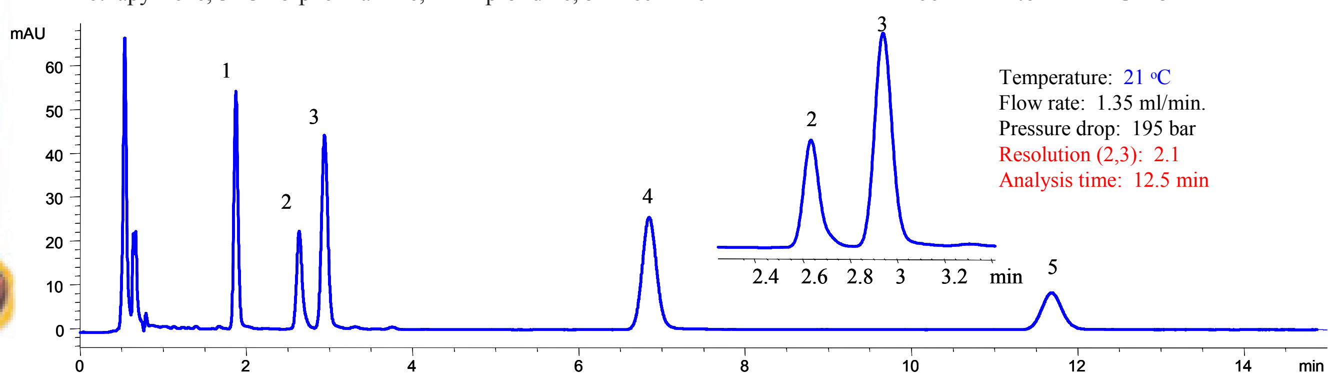
Elevated temperature can allow some separations not possible at ambient temperatures, however the main advantage of using high temperature is faster analysis times. The Metalox® 200-C can help you to increase your laboratory's throughput, sometimes obviating the need to buy additional expensive HPLC systems. The chromatograms in Figure 2 clearly demonstrate the gains in speed (12-fold improvement!) that can be made **without losing resolution** by simply increasing column temperature.

*Figure 2. Analysis of Pharmaceuticals at 21 °C and 140 °C - **12 fold decrease in analysis time.***

LC Conditions: Mobile Phase, 26.5/73.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 1.35 mL/min.; Injection volume, 0.2 ul; 254 nm detection; Column Temperature, 21°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine **100mm x 4.6mm ZirChrom®-PBD**

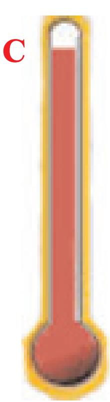
21°C





LC Conditions: Mobile Phase, 20.5/79.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 4.20 mL/min.; Injection volume, 0.5 ul; 254 nm detection; Column Temperature, 140°C; Pressure drop = 194 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine **100mm x 4.6mm ZirChrom®-PBD**

140°C



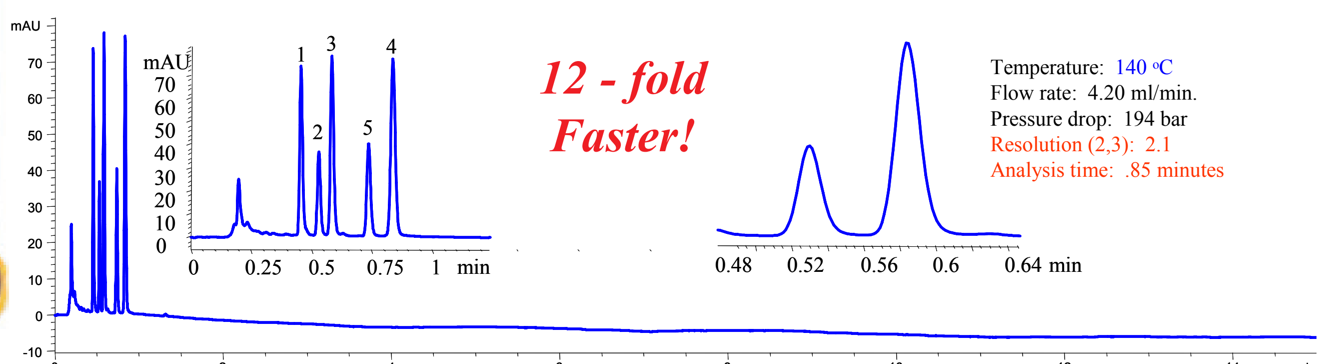


Table 2 is an example of the the final analysis generated by a value assessment program used by ZirChrom® using the data from Figure 2. This assessment can be customized to your specific application to quantitate how temperature can help your productivity. Contact a ZirChrom representative today to help adapt this value assessment model to your specific analysis.

Table 2. Cost Effectiveness of Analysis Time Decrease.

Quantitative Value Assessment
Pharmaceutical Separation
Customer: Contract Lab
Date: 4/25/02

	ZirChrom-PBD	ZirChrom-PBD
Column Cost	$595.00	$595.00
Analysis Time (min)	12.0	1.0
Average Time per Successful Cycle (min)	14.3	3.0
Possible Cycles per Instrument per Year	**6,562**	**30,856**
Cost Components		
Column Cost per Successful Cycle	*$1.35*	*$0.89*
Solvent Cost per Successful Cycle	*$0.17*	*$0.10*
Waste Disposal Cost per Successful Cycle	*$0.04*	*$0.03*
Total Fixed Cost per Successful Cycle	*$3.30*	*$0.74*
Total Operator Cost per Successful Cycle	*$1.68*	*$0.36*
Total Cost per Analysis*	**$6.55**	**$2.12**
Savings per Instrument per Year**	[redacted]	**$136,504**

Note: Calculation does not include costs which are constant for all columns (eg. sample prep) or indirect costs related to the speed of the results.

The Bottom Line: A Case Study

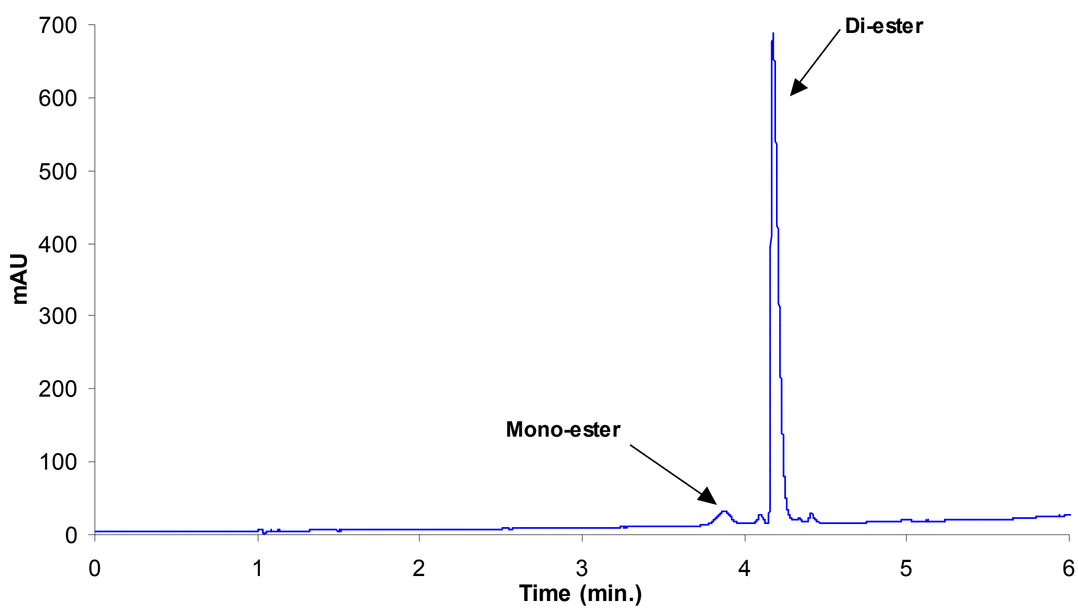
The following case study demonstrates the cost savings that were actually realized by using elevated column temperature. This summary represents a customer experience using the Metalox® 200-C column heater and ZirChrom columns. Surface Specialties UCB is a company located in Smyrna, Georgia who manufacturers specialty chemicals, coating resins, additives, adhesives, and technical resins. Surface Specialties UCB approached ZirChrom method developers with the challenge of shortening the run time of an analysis of mono- to di- ester ratio in a final polymer product. The current methodology for Surface Specialties UCB's analysis of the mono- to di-ester ratio in a final polymer product, specifies an HPLC method which is 30 minutes in length. Two injections are required for each process sample bringing the total analysis time for each process sample to approximately 60 minutes. This analysis is used to monitor in-process reactions to determine completion. Using the current method, the pertinent information regarding reaction completion would be obtained when it was no longer relevant, that is when the reaction had already gone too far.

ZirChrom Solution: ***Use temperature to speed up the analysis!*** Figure 3 shows what ZirChrom® and Metalox® method developers did for the customer's separation.

Customer Allyson Norman, Analytical Scientist with Surface Specialties UCB had this to say about the ZirChrom method; "ZirChrom method developers reduced our analysis turn around time from 1 hour to 10 minutes!"

The ZirChrom value assessment program predicts reducing the total analysis time from 60 minutes (30 minutes/injection) to 10 minutes (5 minute/injection) will save Ms. Norman's company roughly $20.00/sample in total analysis costs. This translates to roughly $158,600/year in savings (savings based on possible cycles/instrument/year using the the ZirChrom column). The savings are after the cost of purchasing the Metalox® 200-C high temperature column heater had been factored into the ZirChrom analysis overhead. Although these cost savings are impressive, and beneficial to the customer, having the analysis completed before the reaction had gone too far is priceless.

Figure 3. Separation of Mono and Di-ester at 80 °C using ZirChrom-PBD and the Metalox® 200-C.



Separation of mono- and di-esters from BPA(EO)30DMA methacrylated polyol. **LC Conditions**: Gradient elution from 20/80 ACN/water to 80/20 ACN/water over 5 minutes; Flow rate, 1.5 ml/min.; Temperature, 80 °C; Injection volume, 5 µl; Analyte concentration is approximately 1 mg/ml; Detection at 240 nm; Backpressure, 155 bar; Column, 150 mm x 4.6 mm i.d. ZirChrom®-PBD

HIGH TEMPERATURE LC BECOMES A REALITY

The innovations of the Metalox® 200-C enables the chromatographer to take advantage of the increased speed of high temperature while eliminating obstacles to high temperature liquid chromatography such as irreproducibility, thermal mismatch band broadening and mobile phase overheating. The revolutionary design of the Metalox® 200-C column heater incorporates a four sensor cascading loop control, a patented active shield adiabatic column jacket and an energy efficient micro heat exchanger (See Figure 4).

Figure 4. Metalox® 200-C.

For more information on high temperature liquid chromatography and full specifications on the Metalox® 200-C high temperature column heater contact Metalox at www.metalox.com or 763-421-3037.

As always, ZirChrom's innovative technical support staff would be happy to assist you in applying this cutting edge technology to your application. Please contact them at support@zirchrom.com or 1-866-STABLE1.

Please visit our website: www.zirchrom.com.

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