Rapid Method Development Using High Temperature HPLC

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Introduction

Extended range HPLC has been investigated for many years; however, progress was delayed because of instrument and column limitations. With the recent introduction of temperature programmable HPLC ovens and columns that are stable to temperatures as high as 200 °C, it is now possible to develop HPLC methods that take advantage of this extended temperature range. A significant increase in speed is the immediately most apparent benefit of elevated temperature under HPLC conditions. However, selectivity changes that can impact separations may also occur. Furthermore, polarity changes occur in reversed-phase solvents. As the temperature increases, aqueous solvents exhibit less polar character. Consequently, programmed temperature gradients can mimic solvent gradients in many cases. In this paper we discuss the use of the new temperature programming techniques in the development of methods to replace the more traditional solvent gradient methods in HPLC.

Instrumentation

High temperature column oven

Selerity Technologies Series 8000 HT/HPLC oven with temperature programming and mobile phase preheating. The Series 8000 is compatible with essentially any HPLC system, so a number of different HPLC systems were used in this study.

Mobile PhasePreheating

- The Key to Successful Extended Range HPLC
  Preheating the mobile phase avoids thermal mismatch within the column. Thermal mismatch will cause significant peak broadening and asymmetry. To avoid dead volume induced performance problems, preheating must be done in a small volume and should be non-invasive. In the Series 8000 and the new Series 9000, the mobile phase is heated within a short, 4-cm zone. Feedback for control is from thermal sensor immediately downstream from the heated zone.

Mobile Phase Preheater*

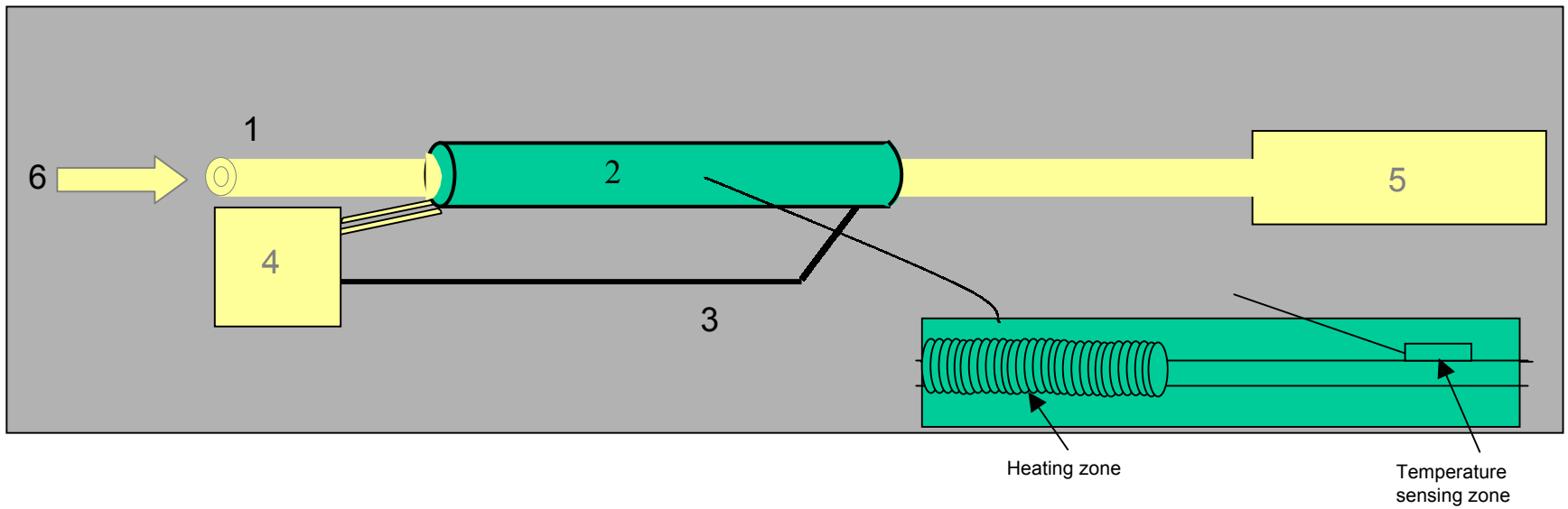


Fig. 1. Schematic of mobile phase preheater: (1) 1/16” OD stainless steel tubing, (2) heater and sensing zone, (3) thermocouple sensor, (4) temperature controller, (5) HPLC column, (6) flow direction from pump

Patent Pending, Selerity Technologies

Steps to Method Development in Enhanced Range HPLC

- Start with mobile phase that is approximately mid range of solvent gradient method
- Set temperature program to run from 40 or 50 °C to 100 °C for Blaze C_8 (silica) column, to 150 °C for polymer or to 200 °C for carbon columns
- Use ramp rate of about 15 °C per minute
- Run in isocratic mode
- Adjust ramp rate and hold times to optimize separation

Developing a Thermal Gradient Method

In this example, we used a mixture of hydrocarbons to demonstrate how a method can be quickly be developed using thermal gradients. From the literature, we recognized that these compounds are typically separated using a solvent gradient of ACN/water. Using this solvent mixture (ACN/water, 25:75), the hydrocarbons were separated at three different temperatures as shown in Figure 2. At the lowest temperature, chrysene takes 22 minutes. At 150 °C, chrysene has a shorter retention time, but now some of the leading compounds co-elute. A temperature gradient was then imposed with the result as shown in the next figure (Fig. 3).

Fig 2. Aromatic Hydrocarbons at Three Temperatures

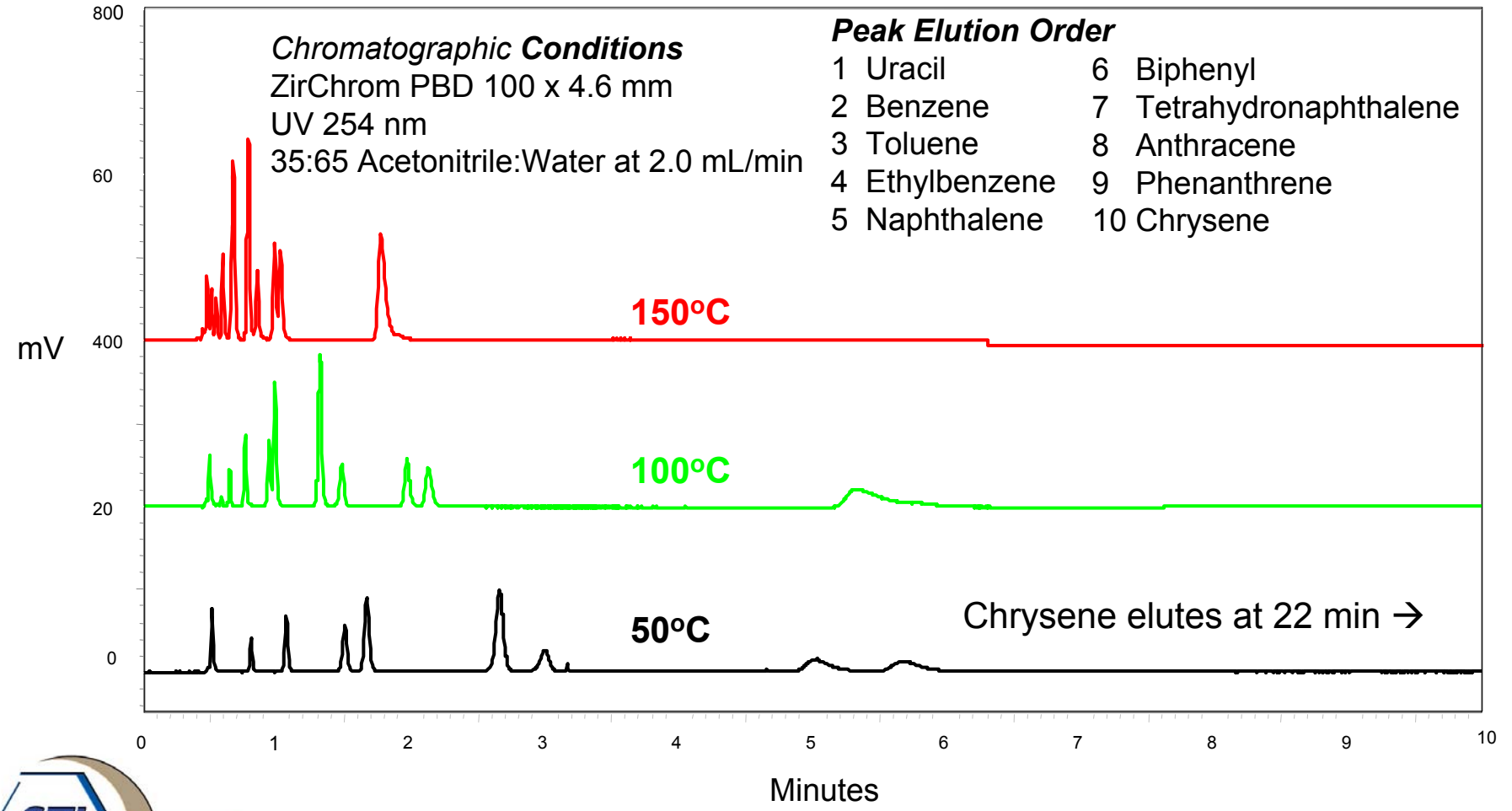
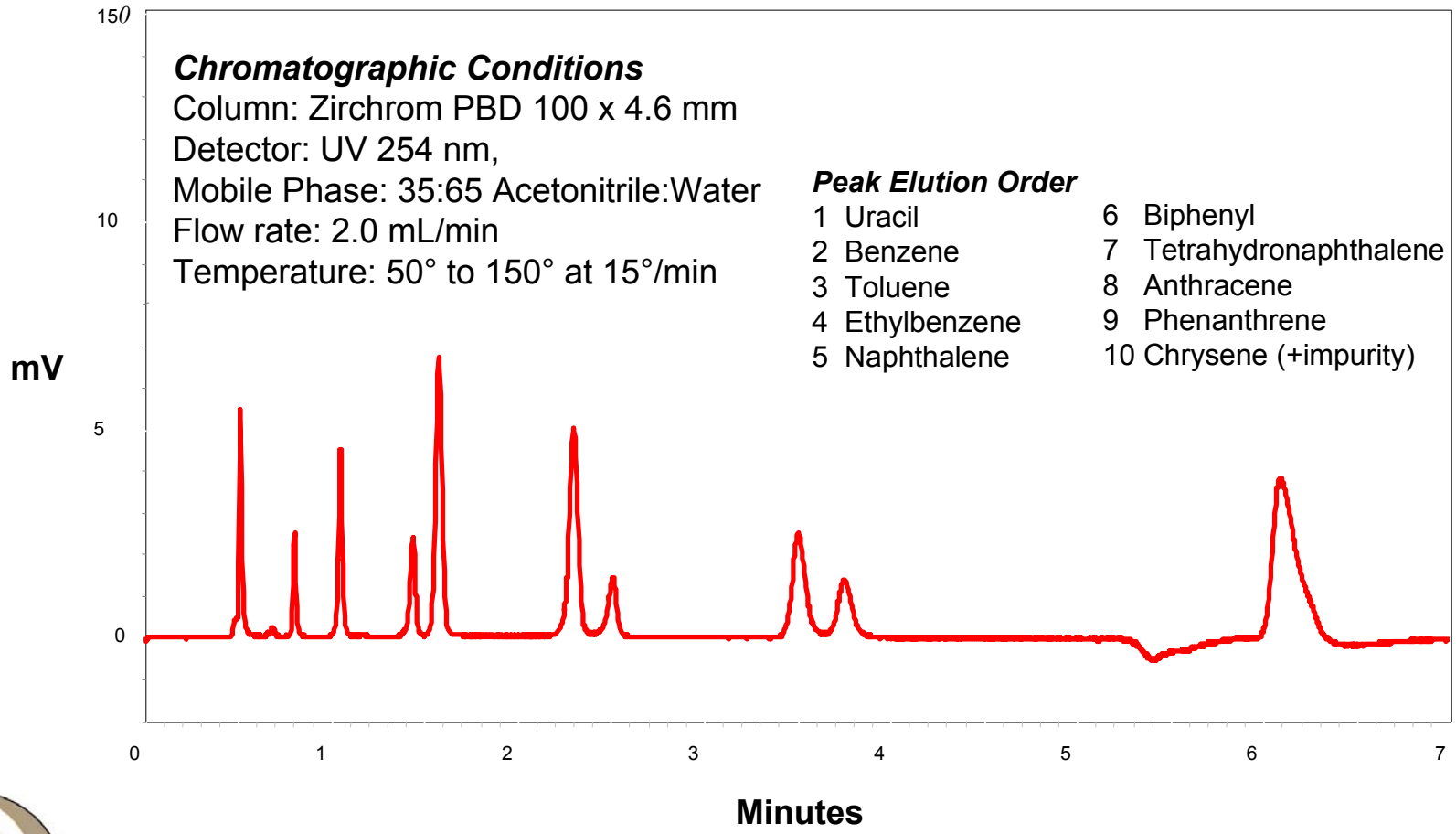


Fig 3. Aromatic Hydrocarbons Using a Temperature Gradient



Natural Product Separations

The following separations typically required run times of about 15 to 20 minutes using an acidic water/methanol gradient (from 80:20 to approximately 60:40 or higher in methanol). Samples were extracted in water/methanol and the extracts injected through a Varian HPLC system. Standards were injected to obtain retention time information: (1)caffeine and theobromine, and (2), catechin and epicatechin. All samples were run isocratically using the same thermal gradient program (40 to 90 °C at 10 °C /min with a Blaze C_8 column with 3-μm particles). Further optimization of the run parameters was not required.

Sample Preparation

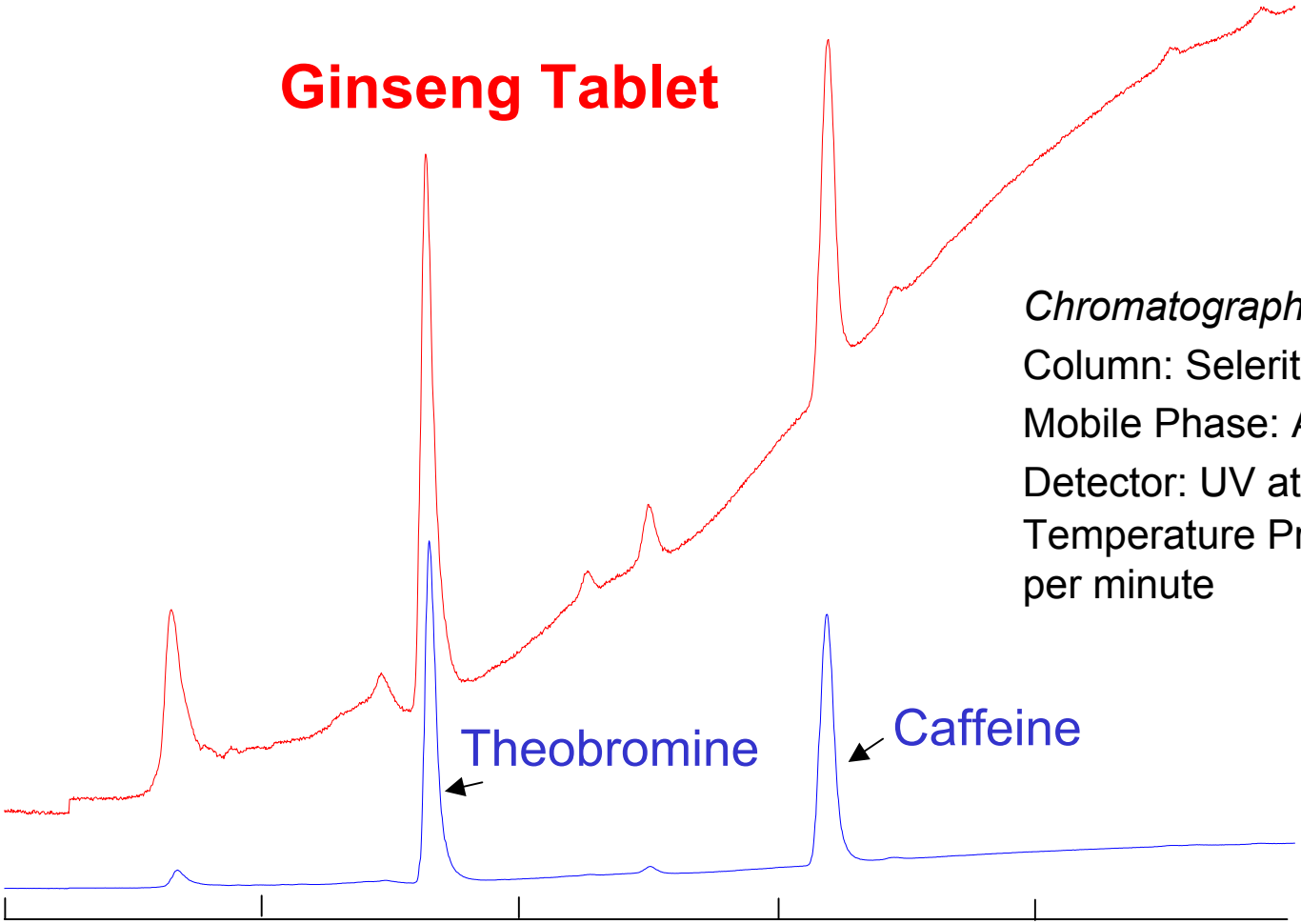
All extracts were done in a mixture of water/methanol at approximately an 80:20 ratio.

Cocoa: Cocoa preparation (Hershey Foods) was extracted as taken from the commercially available source

Ginseng tablet: Tablet contents from a commercial “Energy Enhancing Ginseng” preparation were emptied into beaker and extracted

Green tea was prepared by steeping a commercially available tea bag. In this case the water extract was injected neat

Ginseng Tablet Extract



Chromatographic Conditions

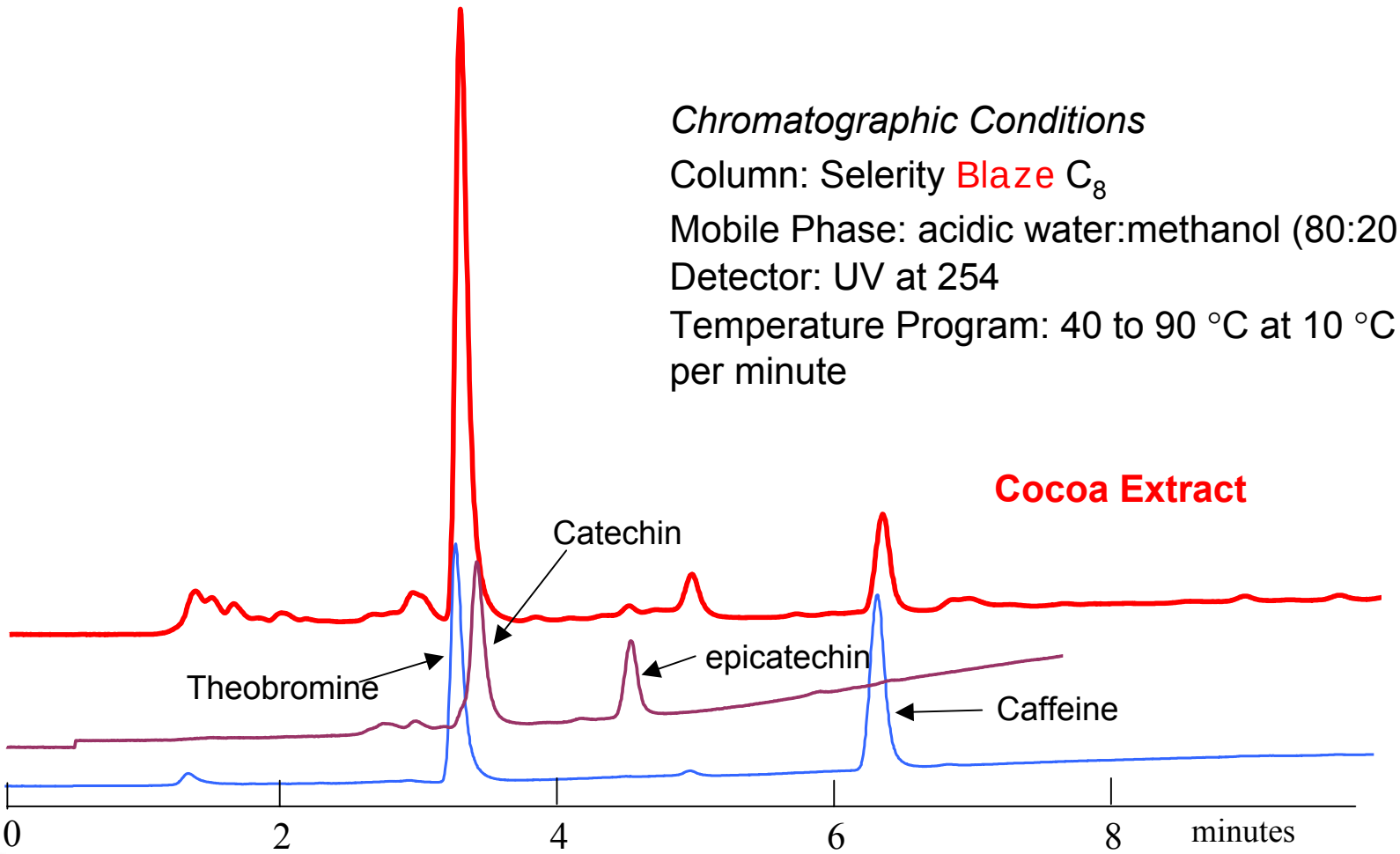
Column: Selerity Blaze C_8

Mobile Phase: Acidic water/MeOH (80:20)

Detector: UV at 254

Temperature Program: 40 to 90 °C at 10 °C per minute

Cocoa Extract



Chromatographic Conditions

Column: Selerity Blaze C_8

Mobile Phase: acidic water:methanol (80:20)

Detector: UV at 254

Temperature Program: 40 to 90 °C at 10 °C per minute

Green Tea Extract

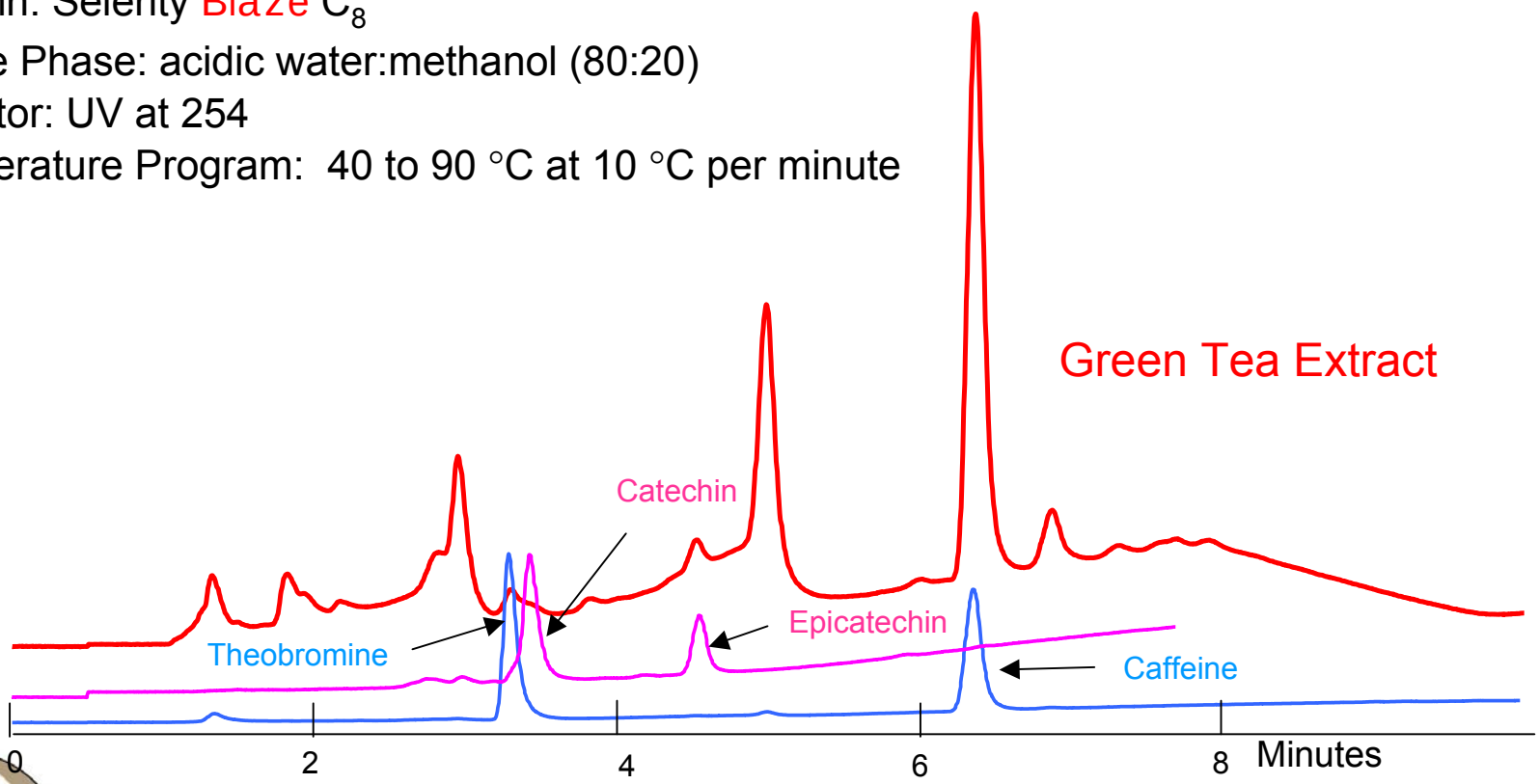
Chromatographic Conditions

Column: Selerity Blaze C_8

Mobile Phase: acidic water:methanol (80:20)

Detector: UV at 254

Temperature Program: 40 to 90 °C at 10 °C per minute



Conclusions

- High temperature HPLC with temperature programming has been demonstrated for a number of complex samples. Method development was rapid and easily performed. Furthermore, temperature gradients have been shown to provide results comparable to solvent gradient HPLC. Temperature gradients are conveniently changed by computer control; consequently, method development is inherently easier than with solvent gradient techniques.

Acknowledgements

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