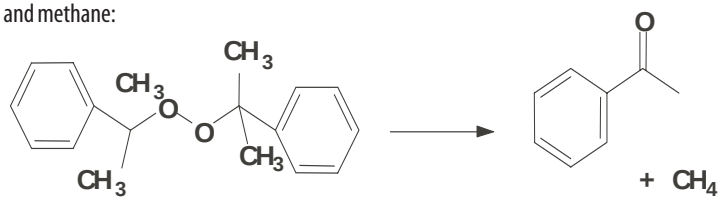
The Effect of Elevated Temperatures on the Thermal Stability of Analytes in High Temperature Liquid Chromatography (HTLC)

Introduction

High Temperature Liquid Chromatography (HTLC) affords shorter analysis times and greater column efficiencies than traditional HPLC analysis. There is a concern, however, that exposure of the sample to elevated temperatures will cause thermal decomposition of the analytes of interest. Questions have also been raised about possible thermal degradation if superheating of the mobile phase takes place within the mobile phase preheater. Dicumyl peroxide (DCP), a dialkyl peroxide that is known to thermally decompose, was analyzed by HTLC to monitor the decomposition at elevated temperatures. Dicumyl peroxide decomposes on heating to produce free radicals, which react to form acetophenone and methane:



Analysis Conditions

HTLC runs at five temperatures and three different flow rates were conducted. Conditions are summarized in Table 1.

Table 1
HTLC Conditions for Analysis of Dicumyl Peroxide

Column:	Zirchrom PDB, 100 x 4.6 mm, 3 µm
Mobile Phase:	40:60 Acetonitrile:Water, isocratic
Flow:	1.0, 2.0 and 4.0 mL/min
Detection:	UV @ 254 nm
Injection:	2.5 µL
Temperature:	90°C, 110°C, 130°C, 150°C and 170°C, isothermal

Table 2
Normalized Percent of Dicumyl Peroxide Remaining

	1.0 mL/min	2.0 mL/min	4.0 mL/min
170 °C	**6%**	**23%**	**45%**
150 °C	**71%**	**77%**	**77%**
130 °C	**88%**	**87%**	**98%**
110 °C	**100%**	**99%**	**98%**
90 °C	**100%**	**100%**	**100%**

Results

Figures 1-3 (see back) show the analyses of dicumyl peroxide at the three different flow rates. Table 2 shows the normalized percents of dicumyl peroxide remaining, calculated from peak areas. No appreciable decomposition of dicumyl peroxide was observed until an analysis temperature of 130 °C. At 170°C, most of the dicumyl peroxide had decomposed. The degree of decomposition decreased as the increased flow rate increased, which is consistent with the premise that it is the time at temperature that is responsible for the extent of breakdown. At 4.0 mL/min, only 55 percent of the dicumyl peroxide had decomposed at 170°C, compared to 94 percent at 1.0 mL/min. In addition, at 170°C and 4.0 mL/min, approximately 6% of the breakdown occurred from the preheater position to the column inlet, with the balance taking place within the body of the column. About 1/3 of this value is due to residence time in tubing and column at 170°C after the preheater. This was determined by measuring the area of the acetophenone peak formed at the beginning of the chromatogram. This was decomposition due to the preheater. The rise in the baseline of the chromatogram is degradation taking place on-column. Drastic superheating of the mobile phase in the preheater would have shifted the decomposition distribution.

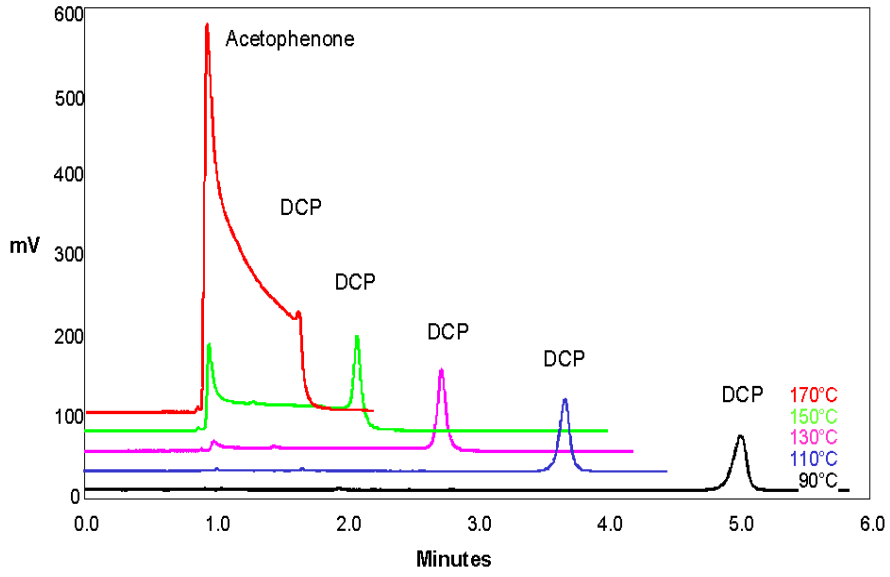


Figure 1: *Decomposition of DCP at 1.0 mL/min*

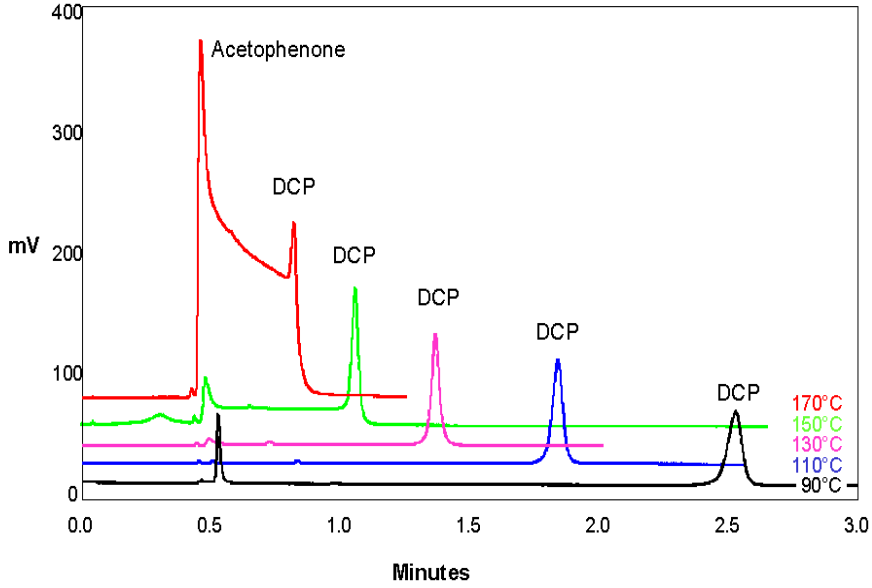


Figure 2: *Decomposition of DCP at 2.0 mL/min*

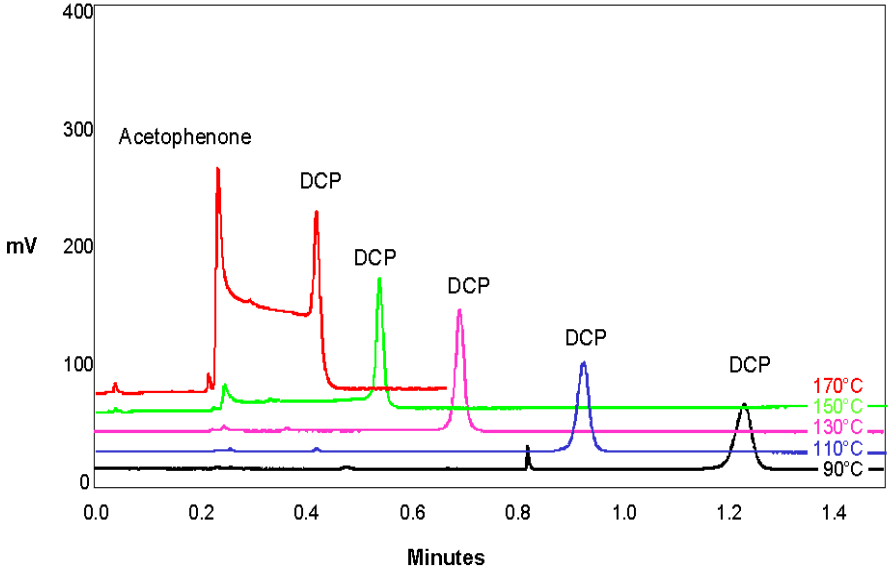


Figure 3: *Decomposition of DCP at 4.0 mL/min*

Conclusions

Dicumyl peroxide was analyzed by HTLC to study the effects of elevated temperatures used in this technique on the decomposition of thermally labile analytes. No significant decomposition of the dicumyl peroxide was observed until 150°C at 4.0 mL /min. Elution of the acetophenone peak shows that the analyte did not completely decompose until 170°C. Because exposure of the analytes to higher temperatures is for a very short time, especially at high flow rates, the risk of decomposition due to elevated temperatures is minimal. Only a small percentage of the degradation occurred due to heating the mobile phase with the Selerity Technologies preheater. Since exposure to high temperatures used in HTLC did not have a major effect on a compound that was known to thermally decompose, it is believed that other analytes will show similar durability under these conditions.