

THE SEPARATION OF STEROIDS BY HIGH TEMPERATURE LIQUID CHROMATOGRAPHY (HTLC) USING WATER AS THE MOBILE PHASE

INTRODUCTION

HPLC analysis at elevated temperatures, with water as a mobile phase, has been shown to replace acetonitrile-water mixtures as an eluent for reversed-phase HPLC. As the temperature increases, the dielectric constant of water decreases while retaining an appreciable density and hydrogen bonding potential. Under high temperature conditions water can be described as a moderate polarity solvent. Furthermore, at high temperatures, the viscosity of the mobile phase is reduced resulting in significantly lower back pressure allowing higher flow rates and shorter analysis times. Higher column efficiencies due to better diffusion rates can improve resolution and sensitivity. In this note a Selerity Series 8000 column oven equipped with a mobile phase preheater and post column cooling was used to obtain a fast separation of steroids. The selection of steroids was separated isothermally at 50°C and 200°C. At 200°C the flow could be increased to 6.0 mL/min and no organic modifier was needed in the mobile phase to perform the analysis.

EXPERIMENTAL CONDITIONS

Conditions are summarized in Table 1. Isothermal runs at 50°C and 200°C were conducted. A Knauer isocratic pump, degasser, and variable wavelength UV detector were used in conjunction with a Selerity Technologies Series 8000 programmable oven.

TABLE 1 : HTLC CONDITIONS FOR ANALYSIS OF STEROIDS	
COLUMN:	ZIRCHROM PBD 100 x 4.6 MM, 3 μM
MOBILE PHASE:	25:75 ACETONITRILE:WATER AT 50°C WATER AT 200°C
FLOW:	3.0 ML/MIN AT 50°C 6.0 ML/MIN AT 200°C
DETECTION:	UV @ 254 NM
INJECTION:	5 μL

RESULTS AND DISCUSSION

Figure 1 shows the separation of steroids at 50°C and 200°C. At 50°C the separation takes less than 1.5 minutes with good peak shape using a 25:75 acetonitrile:water mobile phase and a flow rate of 3.0 mL/min. At 200°C because of reduced viscosity and lower back pressure the flow could be increased to 6.0 mL/min. The separation takes less than 30 seconds with greatly improved efficiency using water as the mobile phase.

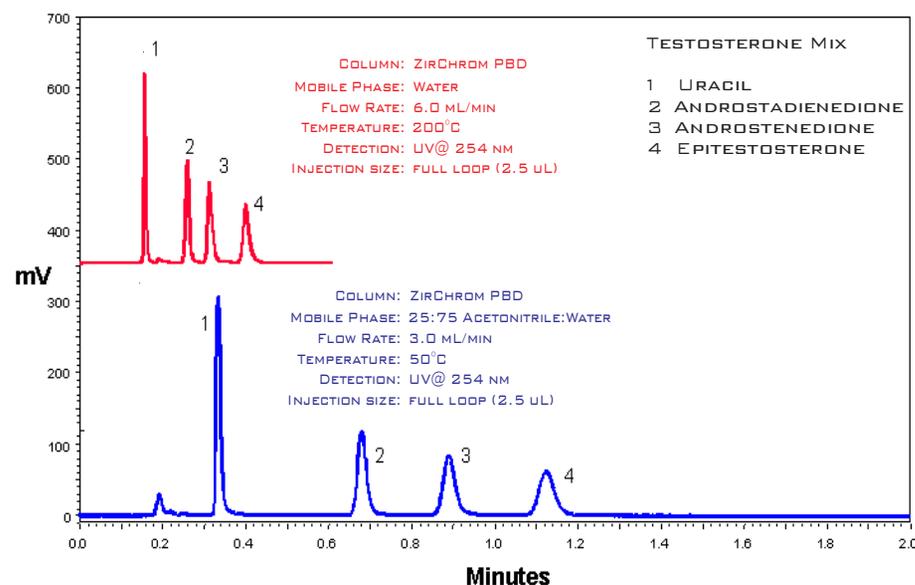


FIGURE 1 : Separation of steroids isothermally at 50°C and 200°C. Note the ability to use a flow rate of 6.0 mL/min and water as the mobile phase at 200°C resulting in a complete separation in less than 30 seconds with increased efficiency.