Separation of Steroid Isomers Using the LaChromUltra HPLC System with UV Detection

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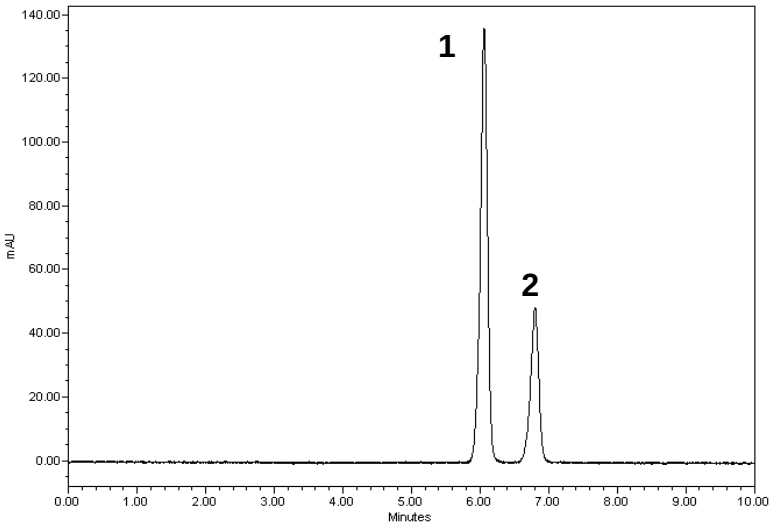
Steroids are a class of molecules with a common fused ring structure that have several key roles in biological systems, and as such, are often used in pharmaceutical applications. Due to the presence of several chiral centers, synthesis of these molecules often results in racemic mixtures of stereoisomers which must be separated to achieve desired product purity. Here we describe a method to analyze the purification products of a racemic mixture of 17α-estradiol and 17β-estradiol following flash separation. These steroid stereoisomers have different pharmacological effects in biological systems. A LaChromUltra HPLC instrument is used to separate these isomers via normal phase chromatography, and separation is monitored by UV absorbance at 228 nm.

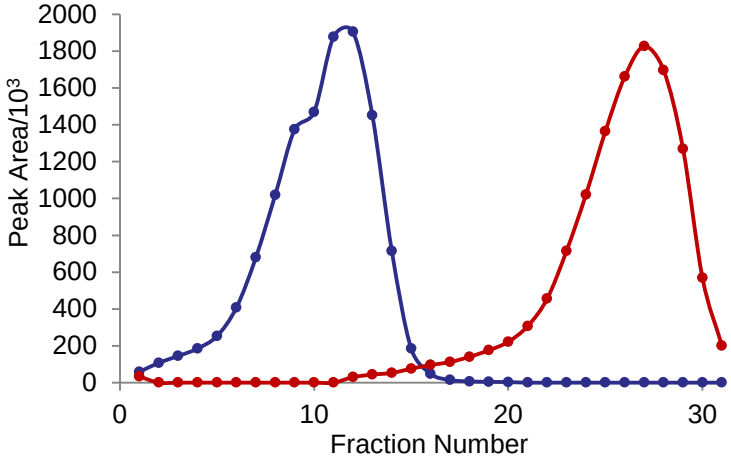
Experimental Conditions

Module	Conditions
Pump (L-2160U)	Mobile Phase: 97% CH_2Cl_2, 3% IPA Flow Rate: 1 mL/min.
Autosampler (L-2200U)	Injection Volume: 5 µL
Oven (L-2300)	Temperature: 25 °C
Detector (L-2400U)	228 nm
Column	Hitachi LaChrom SIL, 5 µm, 4.6 x 250 mm

Results – (1) 17α -estradiol and (2) 17β-estradiol



Results- Analysis of Flash Chromatography Separation of a Racemic Mixture of 17α and 17β-Estradiol



Discussion

Hitachi's LaChromUltra liquid chromatography system with UV detection is effective at rapid analysis of fractions from purification scale chromatography. The percentages of each stereoisomer found in a fraction can be analyzed within 10 minutes.

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