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APPLICATION HIGHLIGHT:
Analysis of Steroids by UltraPerformance Convergence Chromatography

Christopher J. Hudalla and Kenneth J. Fountain/Waters Corporation, Milford, MA, USA

INTRODUCTION

Steroid biosynthesis is a complex metabolic pathway utilizing simple precursors to synthesize multiple steroidal forms. This biosynthetic pathway is unique to animals and provides a common target for antibiotics and other anti-infective drugs. Precise and accurate steroid analysis is critical for the development of steroid-based therapeutics. Typical analysis methods utilize GC/MS, which require sample derivatization and lengthy analysis times (~25 minutes), or LC/MS with typical analysis times of four to 12 minutes. Many of the steroid structures are closely related making their analysis challenging even when using the selectivity of mass spectrometric detection. Chromatographic separation is, therefore, essential for analysis of steroids and steroid derivatives resulting in long analysis times. Convergence chromatography, with CO_2 as the primary mobile phase, presents a unique opportunity to provide rapid and precise analyses for these structurally related compounds, as shown in Figure 1.

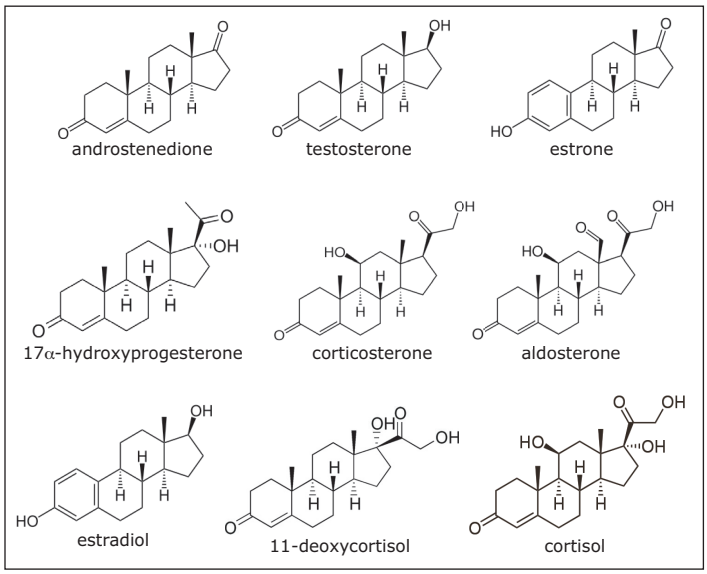

Figure 1. Steroid structures for the current investigation.

EXPERIMENTAL CONDITIONS

A mixture of nine steroids was prepared at a concentration of 0.2 mg/mL each, using a diluent of 88:12 methanol/ethanol. Steroids used included the following: androstenedione, estrone, 17α-hydroxyprogesterone, testosterone, 11-deoxycortisol, estradiol, corticosterone, aldosterone, and cortisol.

All data was collected on an ACQUITY® UltraPerformance Convergence™ Chromatography (UPC²™) System with photodiode array (PDA) detection. The steroid sample was screened on three different ACQUITY UPC²™ column chemistries including: BEH, BEH 2-EP, and CSH Fluoro-Phenyl, using a 1.7 µm particle size in a 3.0 x 50.0 mm column dimension. The mobile phases were CO_2 with methanol as a co-solvent. A two-minute screening gradient was used from 2% to 17% methanol at a flow rate of 3.65 mL/min, and a temperature of 40 °C. The Automatic Back Pressure Regulator (ABPR) was set to 1800 psi. Data was collected at 220 nm (compensated for 380 to 480 nm). The injection volume was 1 µL.

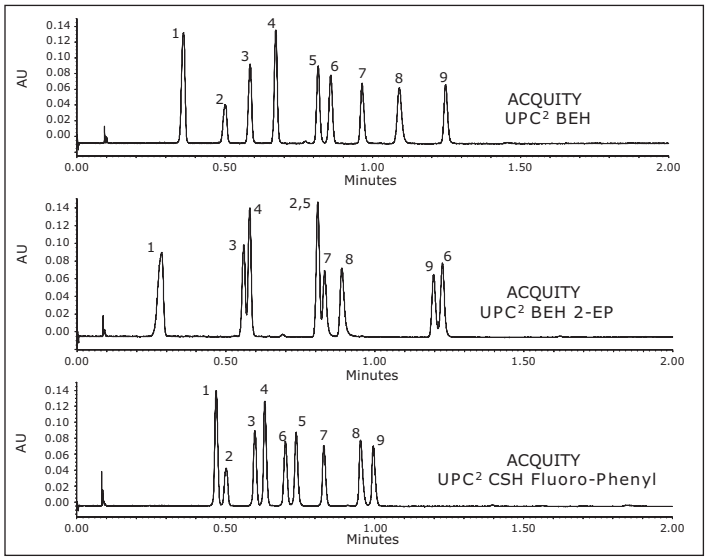

Figure 2. UPC² separations (with UV detection) of steroid standards on the following ACQUITY UPC² columns: BEH (top), BEH 2-EP (middle), and CSH Fluoro-Phenyl (bottom). All columns were 1.7 µm, 3.0 x 50.0 mm configurations. Steroid compounds are (1) androstenedione, (2) estrone, (3) 17α-hydroxyprogesterone, (4) testosterone, (5) 11-deoxycortisol, (6) estradiol, (7) corticosterone, (8) aldosterone, and (9) cortisol.

Click on the underlined blue text for details on the products used in this application.

CONCLUSIONS

The chromatograms shown in Figure 2 demonstrate the selectivity differences of the ACQUITY UPC^2 stationary phases, as well as the inherent speed of this chromatographic technique, with a significant reduction in analysis times compared to alternative techniques. Without the need for derivitization (required for GC analysis), samples can be analyzed directly in organic extraction solvents, omitting the need for diluent exchange for compatibility with reversed-phase LC methods. These factors combined yield a streamlined workflow with significant savings in analysis and sample prep time, solvent costs, and solvent waste disposal.

ORDERING INFORMATION

Columns	Part Number
ACQUITY UPC^2 BEH 3.0 x 50.0 mm, 1.7 µm Column	186006562
ACQUITY UPC^2 BEH 2-EP 3.0 x 50.0 mm, 1.7 µm Column	186006580
ACQUITY UPC^2 CSH Fluoro-Phenyl 3.0 x 50.0 mm, 1.7 µm Column	186006571

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com