Improved HPLC Separation of Steroids

Overview

The chromatograms below demonstrate the complete separation of a mixture of 12 steroids using an Advanced Chromatography Technologies ACE C18-AR column. Under these conditions, ACE C18 and ACE Phenyl columns both show two incompletely resolved critical pairs of peaks. The ACE C18-AR column combines the benefits of the hydrophobic interactions associated with a standard C18 phase with the additional aromatic (π-π) interactions commonly exhibited by Phenyl bonded phases.

HPLC Conditions*

Column: ACE 3 C18-AR (3µm, 150 x 4.6mm)
Mobile Phase: A. Water B. Acetonitrile
Gradient:

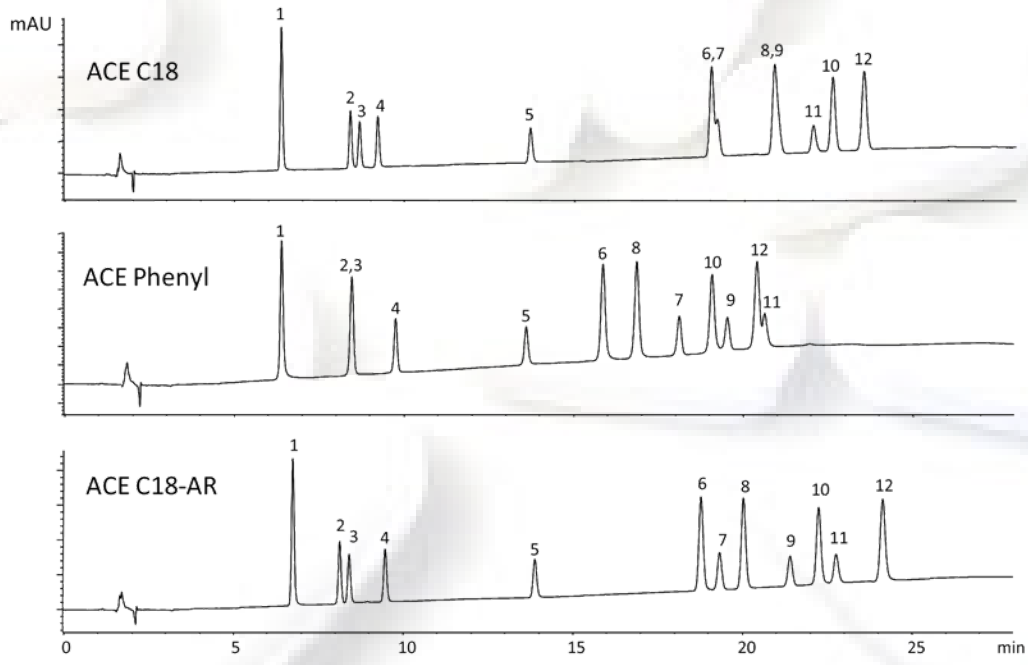
Time (mins)	%B
0	25
24	46
26	46
27	25

Flow Rate: 1.0ml/min
Column Temperature: 20°C
Detection: UV, 214nm
Sample Concentration: 25µg/ml methanol

* Conditions optimised using Drylab 2000 Plus method development software

Separation of 12 Steroid Standards

App No. 1160



1. Estriol
2. Prednisolone
3. Hydrocortisone
4. Cortisone
5. Corticosterone
6. 17β-Estradiol
7. Cortisone-21-acetate
8. 17α-Estradiol
9. 19-Norethindrone
10. 17α-Ethynylestradiol
11. 21-Hydroxyprogesterone
12. Estrone