

CAPSULES

preliminary notes and applications from Bioanalytical Systems, Inc.

High Sensitivity LCUV of Steroids and Nitrocompounds

BAS has recently added a new high performance fixed wavelength absorbance detector (the UV-8) to our LC product line. The unit is fully compatible with the BAS line of LC analyzers. The BAS UV-8 can be configured for virtually any wavelength from 214 to 800 nm. The useable absorbance range is from 0.0005 to 2.0 AUFS.

The performance of the BAS UV-8 absorbance detector was assessed using a mixture of 2 classes of compounds which absorb at 254 nm, steroids and nitroaromatics.

Steroids are important in the treatment of disease, and altered steroid disposition is useful in monitoring steroid-drug interaction. LCUV has been successfully used for the determination of ketosteroids from human serum. Nitroaromatics are commonly incorporated (via derivatization) into the structures of poorly-absorbing analytes to improve (lower) detection limits. In addition, nitroaromatics are the active component of many agricultural chemicals and explosive formulations. Nitrocompounds are, of course, electrochemically active. This presents the possibility of enhanced selectivity using the LCEC/UV combination.

The BAS UV-8 Detector has excellent sensitivity (maximum gain, 0.0005 AUFS) and exhibits improved noise characteristics. Peak-to-peak noise in the baseline is presented in Figure 1 for steroids and nitro compounds. These traces were recorded at 0.001 AUFS using a detector rise time of 3.0 seconds. The calculated peak-to-peak noise in Trace A (LC conditions for steroids) is 3.5×10^{-6} A.U. Trace B, generated under LC conditions suitable for the determination of organic nitro compounds, exhibits peak-to-peak noise of 3.3×10^{-6} A.U. Signal-to-noise ratios for the steroids (Table 1) and nitroaromatics (Table 2) allow for detection limits in the subnanogram range. Representative

chromatograms of steroids and nitroaromatics are presented in Figures 2 and 3, respectively.

NOTE:

The UV-8 Absorbance Detector can be used for conventional, microbore, or preparative LC applications. The detector can be used in tandem with BAS EC detectors with no adverse effect on performance to either detector. Information on other features of the BAS UV-8 Absorbance Detector can be obtained by contacting BAS. The UV-8 is compatible with models LC-150, LC-300 and BAS400 Analyzers.

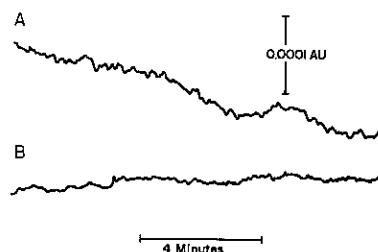


Figure 1. Baseline traces at 254 nm and 0.001 AUFS, using a 3.0 second rise time, of the mobile phase used for steroid (A) and nitroaromatic (B) detection. Other LC conditions given in Figures 2 and 3.

Table 1. Signal-to-noise ratios for steroids. Conditions as given in Figure 2.

Compound	Amount Injected	Signal/Noise
Prednisone	60×10^{-12} g	40.0
Cortisol and Prednisolone*	12×10^{-12} g	40.2
Methyl Prednisolone	60×10^{-12} g	53.0

* co-eluting compounds



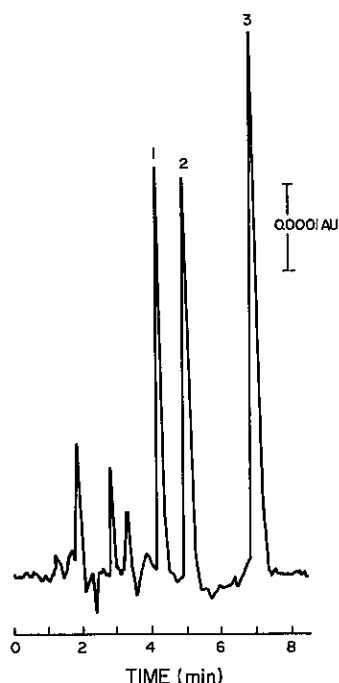


Figure 2. Chromatogram of selected steroids. BAS UV-8 operated at 254 nm, 0.001 AUFS; column, ODS 3 μ m, 100 x 4.6 mm; flow rate, 0.86 mL/min; mobile phase, 55% methanol: 45% H₂O. Peak 1, prednisone; Peak 2, cortisol and prednisolone co-eluting; Peak 3, methyl prednisolone. Amount injected was 200 pg of each steroid.

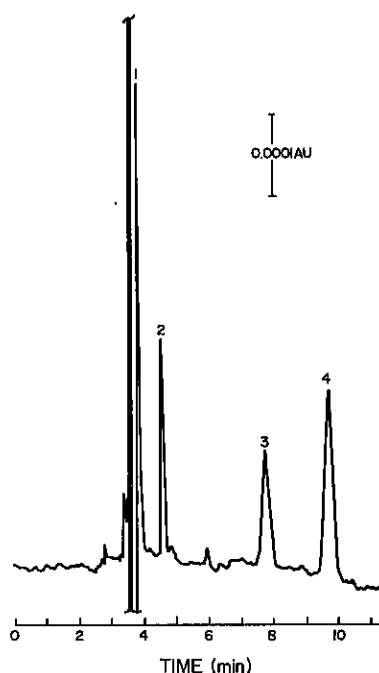


Figure 3. Chromatogram of selected nitroaromatics. BAS UV-8 operated at 254 nm, 0.001 AUFS; column, Biophase Octyl 5 μ m, 100 x 4.6 mm; flow rate, 1.2 mL/min; mobile phase, 80% 0.05 M sodium acetate (pH 5.0): 10% CH₃CN: 10% n-pro-panol. Peak 1, methyl orange; Peak 2, p-nitro-aniline; Peak 3, 2,4-dinitrophenol; Peak 4, 3,4-dinitrotoluene. Amount injected was 200 pg of each nitroaromatic.

Table 2. Signal-to-noise ratio for nitroaromatics. Conditions as given in Figure 3.

Compound	Amount Injected	Signal/Noise
p-Nitroaniline	100 x 10 ⁻¹² g	31.6
2,4-Dinitrophenol	100 x 10 ⁻¹² g	17.3
3,4-Dinitrotoluene	100 x 10 ⁻¹² g	29.1