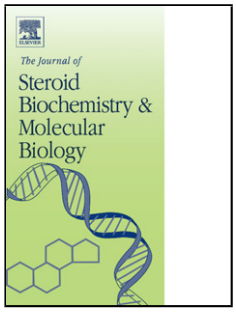

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An improved micro-method for the measurement of steroid profiles by APPI-LC-MS/MS and its use in assessing diurnal effects on steroid concentrations and optimizing the diagnosis and treatment of adrenal insufficiency and CAH.

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Highlights ►
► ► A micro-method for the measurement of a nine steroid profile. ► Micro-method for DHEA and androsterone, a steroid in the androgen backdoor pathway. ► Significant diurnal fluctuation in DHEA concentration from 8am to midnight. ► Time specific reference intervals and universal collection times recommended. ►

Abstract
Our goals were to 1) develop an improved micro-method usable for neonates for steroid profile measurements and a method to measure androsterone, a key steroid in the recently described androgen backdoor pathway together, with dehydroepiandrosterone and 2) to assess if dehydroepiandrosterone diurnal concentration fluctuations exist potentially necessitating strict adherence to time of blood sample draw and requirement of separate time-dependent reference intervals. Liquid chromatography-tandem mass spectrometry was performed with an atmospheric pressure photoionization source [1]. For each sample 50 µL (100 µL for the backdoor pathway) of serum was deproteinized by adding 75 µL (150 µL for the backdoor pathway) of acetonitrile containing the internal standards. After centrifugation, 75 µL (150 µL for the backdoor pathway) of supernatant was diluted with 250 µL of water and injected onto a Poroshell 120 EC-C8 column (SB-C8 column for the backdoor pathway). Within-run coefficients of variation ranged from 2.4-10.4% and between-day coefficients of