Were Your Cortisol Levels Measured Correctly?

Distinguishing Cortisol from Corticosteroids

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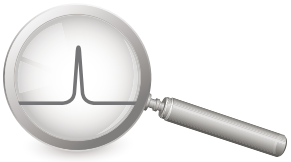
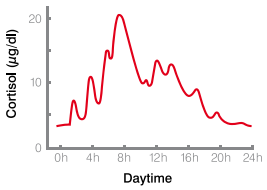
Introduction:

For the determination of the systemic bioavailability of corticosteroids by suppression of the native cortisol plasma level a highly sensitive and selective method is indispensible.
Immunoassays for low levels of cortisol are largely not sensitive enough and most of them are not sufficiently selective.
Various cross-reactions mimic much higher levels of cortisol whereas in reality they are low! The consequence can be fatal.
With the HPLC-MS/MS method described, particularly our HPLC separation, we are able to determine down to 1.5 ng cortisol per mL plasma, serum and urine (even separation of prednisolone, which is not separated by C8 or C18 columns).



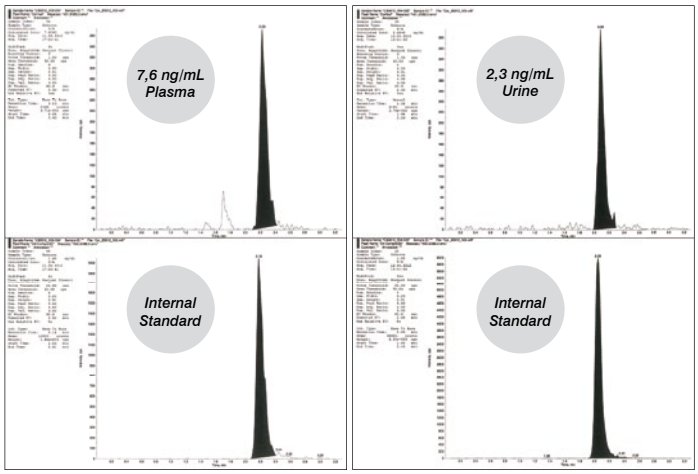
Why is High ***Sensitivity*** Critical?

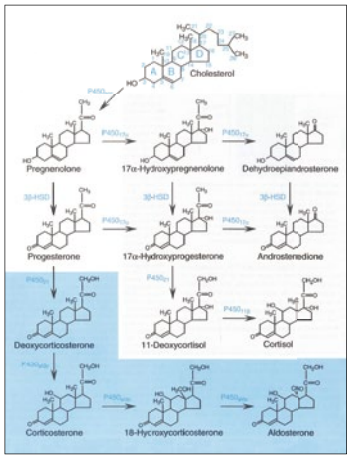
It is the circadian fluctuation of cortisol in combination with corticosteroid suppression of cortisol, that demands the limit of determination very low (1.5 ng/mL plasma / serum / urine).
Calibration range: 1.5 – 500 ng/mL



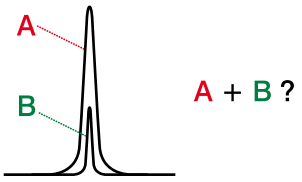
‹ ***Diagram to the left:***
Typical circadian fluctuation of cortisol in blood. (modifidied by Guyton, Textbook of Medical Physiology, 10. Edition., 2000)

Chromatogram: Plasma 7,6 ng/mL /and Urine 2,3 ng/mL





‹ ***Source image on left side:***
„The Pharmalogical Basis of Therapeutics", Goodman & Gilman's , 9th Edition (P1462) Authors: Joel G. Hardman, Lee E. Limbird, Perry B. Molinoff, Raymond W. Ruddon, Alfred Goodman Gilman



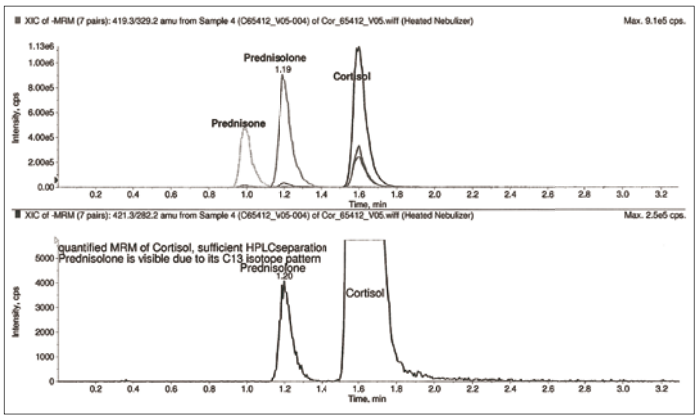
Why is High ***Selectivity*** Critical?

Administering corticosteroids (oral, topical, inhalative) and analyzing by immunoassays, there's the danger of so called "cross-reactions" resulting in wrong (higher) levels. When suppressing cortisol by corticosteroids the levels of native cortisol will decrease and escalate the problem further.
Even using the highly selective HPLC-MS/MS systems, so called "cross-reactions" can occur!
For example, by application of prednisone (attention: metabolite is prednisolone) or initially prednisolone a disturbing peak of the prednisolone in the chromatogram is likely to bias the cortisol results.
In general, on reverse phase columns (C8, C18) prednisolone (MW 360) is not separable from cortisol (MW 362) and consequently adds to the sensitive iontrack of cortisol a signal of identical fragmentation!

1000 ng prednisolon per mL plasma
fakes ca. 10 ng cortisol per mL plasma!

With pharm-analyt's method of separating prednisolon and cortisol (versus C8 or C18) extremely high selectivity can be reached.

Chromatogram of Interference Sample, Good Chromatographic Separation:



Relevance

- *Developing corticosteroids (oral or especially inhalative), new drugs or new galenic formulations.*
- *During clinical studies, monitoring the cortisol suppression of anti-inflammatory anti-asthmatics (phase I/phase II).*
- *MDs who require the "true" cortisol levels of their patients especially at night (demanding higher sensitivity)*