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Background:

Cortisol measurement is useful in evaluation of Cushing syndrome, adrenal insufficiency, mineralocorticoid excess and congenital adrenal hyperplasia. We developed a liquid chromatography–tandem mass spectrometry (LCMS/MS) method with the TQ5500 (ABSciex - Fig. 1) for salivary and urinary cortisol and we determined the 95th percentile (p95) for the urinary (24h collected) and salivary cortisol (morning and evening). We compared them to the Mayo Clinic expected values.

Materials and Methods:

Saliva at 8am and 11 pm and 24h urine were obtained from 32 healthy (22 female, 34.3±9.3 yo) volunteers following informed written consent and approval by the Ethical committee of our institution. We performed validation with the enoval software (Arlenda, Belgium) on 6 and 5 levels that we analysed during 3 days for CORS and CTU respectively. For the validation, we used water or urine with spiked known amounts of cortisol for the CORS and CTU respectively. For the CORS, samples were centrifuged, deuterium labelled cortisol was added as internal standard and the protein precipitated by acetonitril. The supernatant was evaporated, dissolved in methanol acidified with acetic acid and analyzed by LCMS/MS (Fig1). For CTU, samples were centrifuged, deuterium labelled cortisol was added as internal standard and diluted by the ammonium acetate and analyzed by LCMS/MS. At the Mayo Clinic, the expected values were 1-7.5 µg/L at 7 a.m-9 a.m and <1 µg/L at 11 p.m-midnight for CORS and 3.5-45 µg/24h for the adults (<18yo) for CTU.

Figure 1

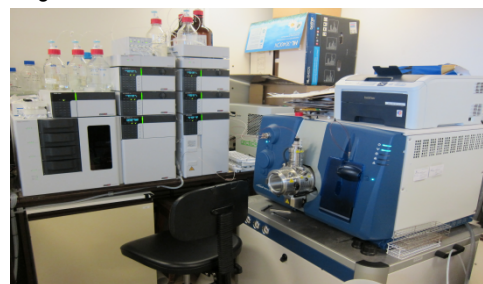


Figure 2

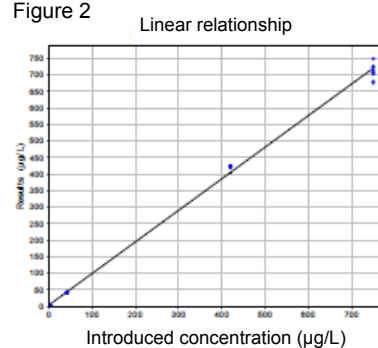
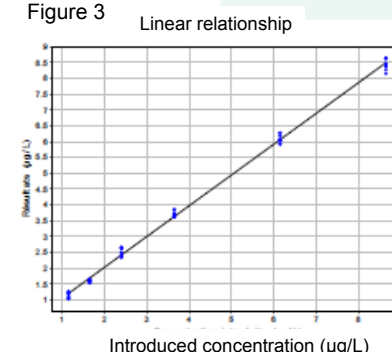


Figure 3



Results:

For the CTU, the with-in run did not exceed 3% (0.4-3%) and the between-run did not exceed 3.1% (0.4-3.1%) in the concentration range 1.5-750 µg/L. The limit of quantification was 1.5 µg/L. The linearity was good between 1.5 and 750 µg/L (Fig 2). The recovery is 97.9±2.2% (95%CI for the mean: 92.4-101.1%). For the CORS, the with-in run and between run did not exceed 8% (1.9-8%) in the concentration range 1.15-8.65 µg/L. The limit of quantification was 1.15 µg/L. The analyse present a good linearity between 1.15 and 8.65 µg/L (Fig 3). The recovery is 99.9±2.9% (95%CI for the mean: 94.2-108.7%). The p95 for the CTU according to the CLSI C28-A3 was 33 µg/24h, and for the CORS were 5.42 µg/L at 8 am and 0.7 µg/L at 12 pm.

Conclusions:

Our developed method in liquid chromatography tandem mass spectrometry was validated for the measurement of urinary and salivary cortisol. Our findings indicate that the proposed analytical methods were suitable for routine purposes and useful in many pathological conditions. The expected values confirm these defined by the Mayo Clinic.