Introduction and Aim

Cortisol measurement is a key-test in the diagnosis of Cushing syndrome [1]. It can be performed in different biological samples ranging from serum to urine and, in more recent years, saliva [2]. In salivary measurements only free hormone is detected, samples can be collected during normal daily routine and stress-induced cortisol release is less likely to occur than during venipuncture. Although immunometric assays for salivary cortisol are available and employed, they suffer from non-specificity due to cross-reactivity with steroid metabolites, synthetic corticosteroids or drugs and lack of standardization between labs [3]. Moreover, the simultaneous determination of salivary cortisol and cortisone may also help clinicians in diagnosing a variety of endocrinological disorders. In collaboration with an industrial Partner (B.S.N. Srl Biological Sales Network, Castelletone, Cremona, Italy), we developed and validated an analytical method for routine measurement of cortisol and cortisone in salivary samples.

Methods

Saliva can be obtained by standard procedures using available devices (Salivette). 200 µl were added to an equal volume of dilution solution (containing cortisol-D4 as internal standard) and 1 ml of extraction solution. After extensive vortexing and centrifugation, 800 µl of the upper phase are collected, dried under nitrogen and resuspended with 30 µl of the reconstitution solution and 30 µl of the dilution solution. After vortexing and resuspension, 60 µl were transferred in a new vial placed in the LC-MS/MS autosampler. Samples were then analyzed using an AB SCIEX Triple Quad™ 6500 LC-MS/MS system (column: Hypersil Gold, ID 2.1x50 mm, 1.9 µm; flow: gradient, as reported in Table I; T 30°C; inj vol: 10 µl; MRM: cortisol: 363.1→121.1, 363.1→105.1; cortisone: 361.1→121.1, 361.1→105.1; cortisol-D4: 367.1→121.1, 367.1→97.1; ESI+). All other MS parameters are reported in Table II.

Results

The validated method displayed as LLOD 5.7 pg/ml (cortisol) and 2.7 pg/ml (cortisone), as LLOQ 19.1 pg/ml (cortisol) and 9.0 pg/ml (cortisone), linearity 19.1-50000 pg/ml (cortisol) and 9.0-160000 pg/ml (cortisone), recovery 96-103% (cortisol) and 89-110% (cortisone); within-run, between-run and total repeatability was respectively 3.7 %, 4.9 % and 6.1 % for cortisol (at a concentration of 15 ng/ml) and 2.5 %, 5.3 % and 5.9 % for cortisone (at a concentration of 38 ng/ml). Samples can be stored up to three months at 4°C or up to one year at -20°C.

Conclusions

A LC-MS/MS method was developed and validated for the routine measurement of cortisol and cortisone in salivary samples. This method will allow to reduce laboratory errors due to both sample collection and unspecific measures, may assist the clinicians in the diagnosis of endocrinological diseases and will avoid unnecessary hospitalization (for late night collection) and invasive procedures.

References