Wednesday, 15:30
Exhibit Hall
Poster #6b

Determination Of Cortisol And Cortisone In Urine By LC-MS/MS: Impact On Quality Results And Cost Benefits

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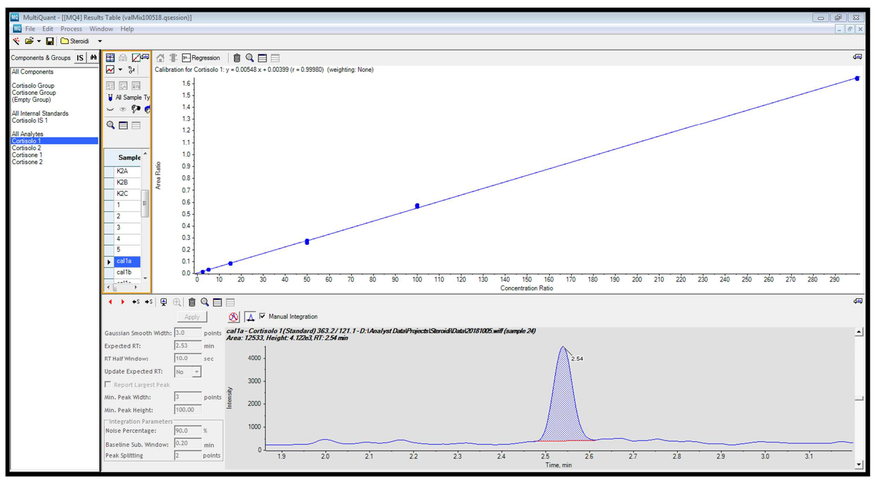

Figure 1. Calibration curves of cortisol (upper panel) and cortisone (lower panel) in LC-MS/MS.

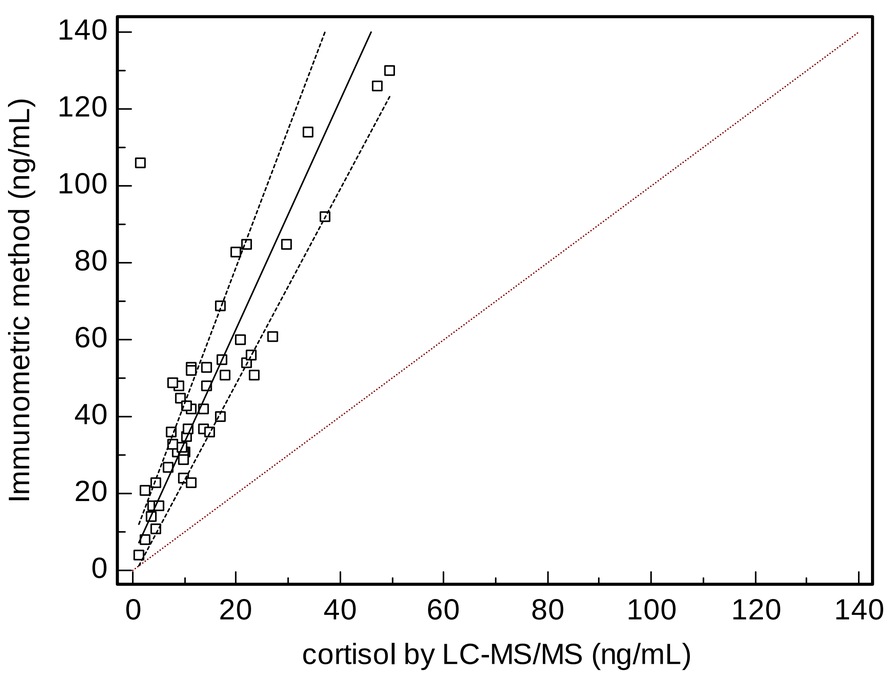

Figure 2. Passing-Bablok regression (X: LC-MS/MS, Y=immunometric method) for only cortisol (upper panel) or cortisol+cortisone (lower panel).

Introduction and Aim

The 24-h urinary free cortisol measurement is widely used as first laboratory approach in the diagnosis of Cushing syndrome and for assessing 11 beta-hydroxysteroid dehydrogenase activity [1-2]. In many labs urinary cortisol is measured by immunometric assay; however, despite technical ease and availability of commercial reagents, these methods suffer from non-specificity due to cross reactivity with steroid metabolites, synthetic corticosteroids or drugs, lack of standardization between labs, personnel and time costs due to sample preparation requiring solvent extraction [2]. Moreover, the simultaneous determination of urinary cortisol and cortisone may also help clinicians in the diagnosis of other endocrinological disorders. In collaboration with an industrial Partner (B.S.N. Srl Biological Sales Network, Castelleone, Cremona, Italy), we developed and validated an analytical method for the routine measurement of cortisol and cortisone in urine samples.

Methods

A 10-mL aliquot was taken out from the 24-h collected urine and stored at -20°C until analysis. Samples were thawed at room temperature and centrifuged at 3500rpm for 10 min. 40µL of sample surnatant, controls (3 levels) and calibrators (6 levels) were then mixed for 30s with 200µL of a working solution (195µl of precipitating agent + 5µL of internal standard cortisol-D4). After centrifugation (5min at 11000rpm), 150µL of surnatant were transferred in a new vial placed in the LC-MS/MS autosampler. Samples were then analysed using an AB SCIEX Triple Quad™ 6500 LC-MS/MS system (column: C18, ID 2.1x50mm, 1.8µm; flow: 0.35mL/min; T 30°C; inj vol: 3µL; MRM: cortisol: 363.2>121.1, 363.2>105.1; cortisone 361.2>163.1, 361.2>121.1; cortisol-D4: 367.2>121.1; ESI+). Within-, between-run and within-lab repeatability were calculated by ANOVA. Method comparison study (Chemiluminescent immunoassay vs LC-MS/MS), evaluated by Passing-Bablok regression and Bland-Altman analysis, was performed using 50 routine samples with cortisol concentrations within the reportable range.

Results

The validated method displayed as LLOD 0.8ng/mL (cortisol) and 0.7ng/mL (cortisone), as LLOQ 2.4ng/mL (cortisol) and 2.1ng/mL (cortisone), linearity 2.5-300ng/mL (cortisol) and 2.5-300ng/mL (cortisone), recovery 87-106% (cortisol) and 80-112% (cortisone); within-run, between-run and within-laboratory repeatability was respectively 6.6%, 5.4% and 8.6% for cortisol and 4.9%, 6.3% and 8% for cortisone (Figure 1). Method comparison study was performed using 50 routine samples. Median (IQR, min-max) cortisol values were 42.0ng/mL (30.5-55.3, 4.0-130) for immunometric method and 11.2ng/mL (7.7-18.3, 1.1-49.6) for LC-MS/MS. Passing-Bablok regression (X: LC-MS/MS, Y: immunometric) showed a remarkable significant systematic proportional error (slope: 2.97; 95%CI 2.52-3.56) (Figure 2, upper panel). Mean %bias was -110% (95%CI from -116 to -103%) with 95% of the differences ranging from -155 to -64%. Interestingly, when cortisol values measured by immunometric method were compared with total (cortisol+cortisone) values measured by LC-MS/MS, neither systematic proportional (slope: 1.03; 95%CI 0.80-1.33) nor constant error (intercept: -6.34; 95%CI -16.86-3.50) nor significant %bias (7.2%, 95%CI -4.9-19.2) were detected (Figure 2, lower panel). With regard to resource costs, the new LC-MS/MS method, avoiding complex sample preparation (extraction, evaporation and sample reconstitution), allows to significantly reduce laboratory errors, personnel and time costs.

Conclusions

A simple LC-MS/MS method was developed for the routine measurement of cortisol and cortisone in urine samples. The new method allows reducing laboratory errors, labor time saving and may assist the clinicians in the diagnosis of other endocrinological diseases.

References

[1] Reimondo G et al. Clin Chim Acta 2008;388:5–14

[2] Antonelli G et al. CCLM 2014;52:213-220