SEX HORMONE AND STEROID PRECURSOR MEASUREMENT BY SIMPLE AND RAPID LC-MS/MS: COMPARISON WITH CURRENT ROUTINE IMMUNOASSAYS

INTRODUCTION

Sex steroid testing has a central role in clinical decision-making and in research studies on diseases such as female hyperandrogenism, male hypogonadism or in inborn disorders of steroid synthesis. In the last decade, LC-MS/MS clearly displayed its analytical superiority over routine immunnoassays (IA) in accurately and sensivity determining serum testosterone and in assessing large androgen profiles, thus promoting studies on the re-characterization of androgen imbalance diseases. Nonetheless, the measurement of other sex steroids and precursors is still challenging, and is available only in experienced research laboratories. Our aim was to develop a simple-prep and rapid LC-MS/MS method for the simultaneous measurement of estrone (E1), estradiol (E2), dihydrotestosterone (DHT) and 17OHprogesterone (OHp).

RESULTS

LC-MS/MS PERFORMANCE AND VALIDATION

REFERENCE INTERVALS: gender and age specific reference intervals were generated on 366 adult normal weight, healthy and drug free volunteers.

FEMALES PRE-MENOPAUSAL

FEMALES MENOPAUSAL

MALES

LC-MS/MS vs IA COMPARISON STUDY

The novel LC-MS/MS assay was compared with IAs currently used by the Central Laboratory of S.Orsola – Malpighi Hospital of Bologna for routine E1 (direct RIA, DSL 8700 by Beckman Coulter) and E2 (ECLIA, Modular II by Roche) assessment (Fig. 1).

CONCLUSIONS

The proposed LC-MS/MS assay showed optimal performance in sensitive and accurately determining routinely-assayed estrogens, confirming the reliability of new-generation ECLIA by Roche for E2 and highlighting the severe unreliability of the direct RIA in determining E1. Our LC-MS/MS method further allowed the determination of important though not-routinely assayed DHT and 17OHpregnenolone. The low bench- and run-time required by the proposed LC-MS/MS assay, together with the reference intervals specific for age, gender and fertility status we provided, allow the immediate application of this powerful technique in research and in clinical settings, for the improvement of the characterization and of the diagnostic efficiency of female hyperandrogenism and male hypogonadism disorders.

Figure 1: Passing & Bablok regression in the overall circulating range and in the lower circulating range.