A 20-Steroid Plasma Panel Based on LC-MS/MS in Endocrine Diseases Diagnosis

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Introduction

Plasma steroids play an important role in the clinical evaluation of a number of common endocrine disorders. Without effective clinical screening, a large part of the endocrine disease patients would lose the opportunity for getting the best results of treatment through drug or surgery. What’s worse, a large number of patients may be exposed in higher risks such as cardiovascular diseases, cerebrovascular diseases and kidney disease. As metabolism is a complex regulatory process, normal terminal product of metabolic pathway like cortisol or aldosterone may result in the miss diagnosis.

The middle products in metabolic pathway (fig 1) must be systematically observed to determine the occurrence and development of the disease. This may be benefit to the accurate diagnosis of endocrine diseases for further accurate and effective treatment.

Method Validation

To validate the developed solid phase extraction (SPE) LC-MS/MS assay, after exclusion of matrix effects and sample interference, the sensitivity, specificity, precision, accuracy, repeatability, intra-batch/inter-assay, recovery, linearity, and lower limits of quantification (LLoQ) were determined in accordance with Chineses guide for liquid chromatography and mass spectrometry clinical application and US FDA for Bioanalytical Method Validation Guidance for Industry. (https://www.fda.gov) Statistical analysis was performed using excel to calculate mean, standard deviation, coefficient of variation and recovery.

Clinical Application

This detection was applied to endocrine diseases diagnosis such as Cushing’s syndrome, adrenal hyperplasia, adrenal gland, pituitary tumor, polycystic ovary syndrome and so on. Our data suggested that monitoring steroid hormone metabolism pathway was indeed benefit for diagnosis.

Method Development

An Waters® Xevo™ TQS MS ACQUITY UPLC® System was used to develop the detection method based on the steroids’ physical and chemical properties(fig 2).

Based on LC-MS/MS technology, 20 steroid hormones including: Testosterone, Androstenedione, Cortisol, 17α-Hydroxyprogesterone, 11-Deoxycortisol, and so on were quantified. 15 analytes were performed in positive ion while 5 analytes were in negative ion mode for within 7 min. The isomers were able to be identified respectively, such as 17-OHP and 21-OHP.(fig 3&4)

Conclusion

We developed a high sensitive, high specific LC-MS/MS based steroid hormones detection which included 20 steroids. This detection was applied to endocrine diseases diagnosis such as Cushing’s syndrome, adrenal hyperplasia, adrenal gland, pituitary tumor, polycystic ovary syndrome and so on.