High-Throughput LC-MS/MS Measurement of pregnenolone in Human Blood Serum for Research Purposes

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ABSTRACT
We developed a LC-MS/MS method for research to accurately measure pregnenolone in blood serum from 10 to 500 ng/dL with a throughput of at least 10 injections per hour. This was done using liquid chromatography coupled to tandem MS analysis using a 4-channel high performance liquid chromatography (HPLC) system coupled to a Thermo Scientific™ Transcend™ LX-4 quadrupole-Orbitrap Mass Spectrometer. A HESI source was used to optimize the ionization of the steroids. Optimization of the method was performed using a peak area surrogate for pregnenolone. For an injection of standard at 300 ng/dL, the area peak for pregnenolone obtained was 250000. The linearity of the method was determined by analyzing standards at four concentrations: 10, 50, 100 and 200 ng/dL. The correlation coefficients were >0.99, with a slope of 1.027, intercept of -0.561 and r² of 0.999. The accuracy of the method was determined by analyzing samples that were tracer spiked with pregnenolone, the average recovery was 94 ± 4.7%. The within and between-batch variabilities were less than 5 and <10%, respectively. The assay was able to measure the pregnenolone levels in blood serum from patients with low levels of the steroid and was able to provide quantitation with a coefficient of variation of less than 5%. The developed method was able to achieve a good correlation with the samples analyzed and was able to provide accurate results.

INTRODUCTION
Pregnenolone is a biologically precursor to other steroids such as corticosteroids, androgens and estrogens. It is converted to progesterone by 3-β-hydroxysteroid dehydrogenase or to 17α-21α-21,21,21α-trihydroxy-4-ene-3,20-dione (D4) by hydroxysteroid dehydrogenase or to 17α-hydroxyprogesterone by 17α-hydroxylase. Pregnenolone can be accurately measured in blood serum by this method which achieved: (a) Analysis range from 10 to 500 ng/dL. (b) Throughputs of 13 or 26 or 52 injections per hour. All injections were analyzed using the same MS/MS conditions. (c) Quantitative results showed good accuracy and precision. (d) The throughput was increased to 26, 39 and 52 injections per hour, respectively. Pregnenolone batches were also run in parallel with enzyme blanks which yielded the same MS/MS structures.

MATERIALS AND METHODS
Sample Preparation
All samples were prepared using a four-channel liquid chromatography system. The sample preparation was performed using a peak area surrogate for pregnenolone. For an injection of standard at 300 ng/dL, the area peak for pregnenolone obtained was 250000. The linearity of the method was determined by analyzing standards at four concentrations: 10, 50, 100 and 200 ng/dL. The correlation coefficients were >0.99, with a slope of 1.027, intercept of -0.561 and r² of 0.999. The accuracy of the method was determined by analyzing samples that were tracer spiked with pregnenolone, the average recovery was 94 ± 4.7%. The within and between-batch variabilities were less than 5 and <10%, respectively. The assay was able to measure the pregnenolone levels in blood serum from patients with low levels of the steroid and was able to provide quantitation with a coefficient of variation of less than 5%.

RESULTS
Quantitation Reliability
Typical results are shown in Table 1. The correlation coefficients were greater than 0.99, with a slope of 1.080, intercept of -0.9706 and r² of 0.9706, respectively. The accuracy assessment showed that the method was accurate with an average recovery of 90% and a coefficient of variation less than 5%. The within and between-batch variabilities were less than 5 and <10%, respectively. (Tables 1a & 1b).

CONCLUSIONS
Pregnenolone can be accurately measured in blood serum by this method which achieved: (a) Analysis range from 10 to 500 ng/dL. (b) Throughputs of 13, 26 or 52 injections per hour and 4 or 8 channel systems. (c) Quantitative results showed good accuracy and precision. (d) The throughput was increased to 26, 39 and 52 injections per hour, respectively. (e) Method performance with other methods utilizing the same HESI source.

REFERENCES

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