Evaluation of a bracketing calibration-based isotope dilution liquid chromatography-tandem mass spectrometry candidate reference measurement procedure for 17α-hydroxyprogesterone in human plasma

Qiaoxuan Zhang[1*], Liqiao Han[1], Jun Yan[1], Jianbing Wang[1], Haibiao Lin[1], Peifeng Ke[1], Junhua Zhuang[1], Xianzhang Huang[1]

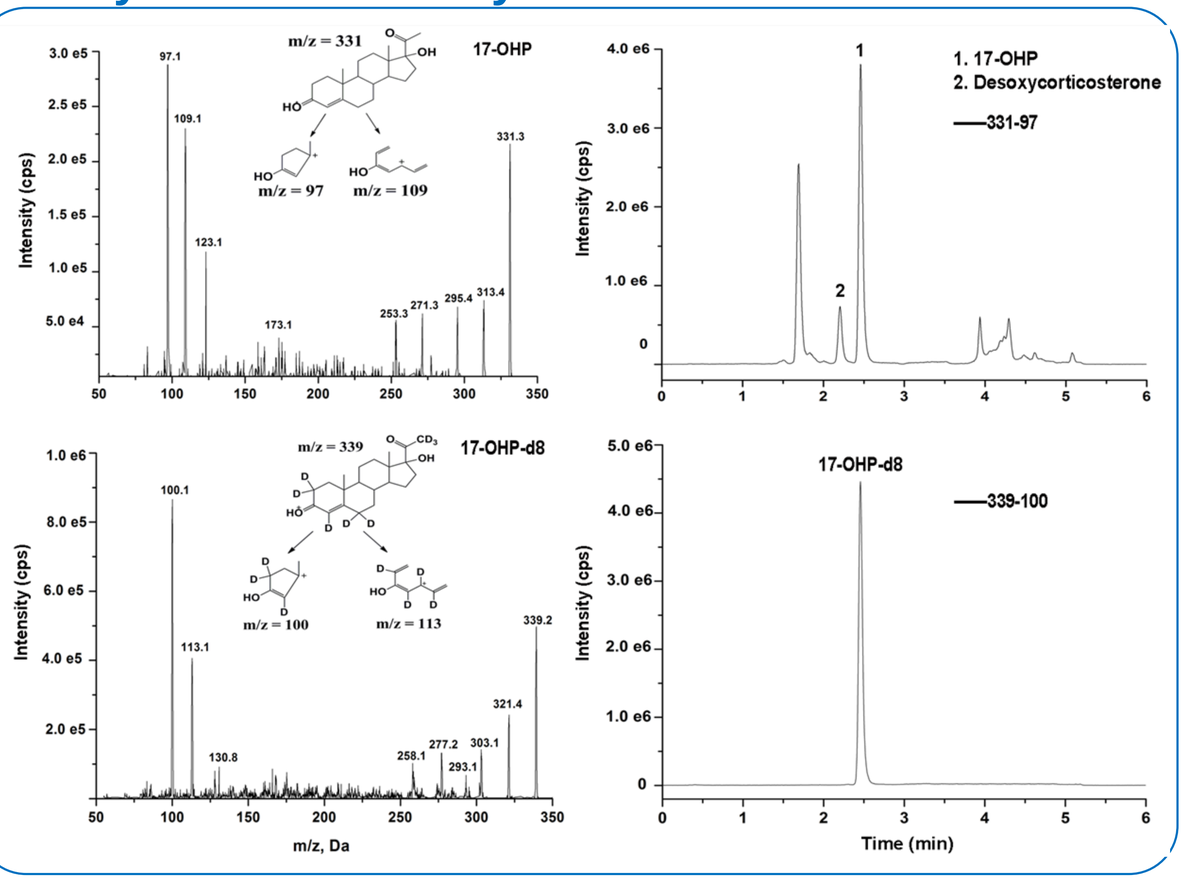
[1] Department of Laboratory Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, 510120, China.

INTRODUCTION

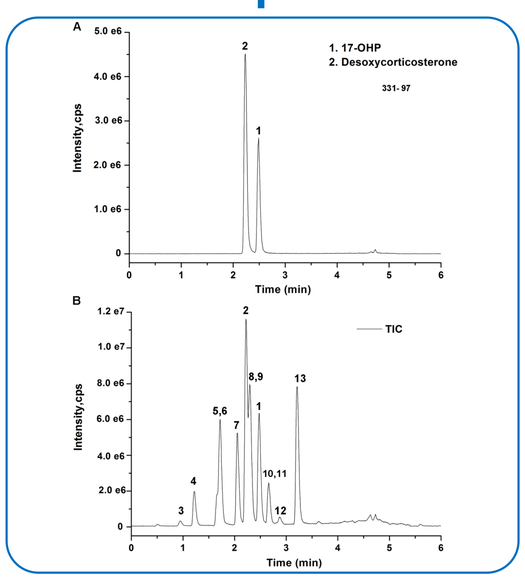
17α-hydroxyprogesterone (17-OHP) is a C-21 endogenous progestrogen which produced during the glucocorticoids and steroids biosynthesis. 17-OHP measurements are reported to be inaccurate and highly variable by use of immunoassay methods. The levels of 17-OHP were always overestimated in preterm infants, creating false-positive reports. To improve accuracy and meet the requirements of 17-OHP measurement in clinical setting, a more sensitivity and specificity method which could reduce parental stress resulting from false-positive screening results is in urgent need. A specific, accurate, reliable and highly sensitive ID-LC-MS/MS candidate RMP (cRMP) for the quantitation of 17-OHP in human plasma by liquid–liquid extraction with n-hexane/ethyl acetate (3:2, v/v) was developed. To achieve higher precision and accuracy, the equilibration of 17-OHP in plasma with a spiked internal standard (IS) was evaluated in the sample preparation, and bracket calibrator was used to assign plasma value. Chromatographic separation of 17-OHP from its analogues was demonstrated. Accuracy was evaluated by comparing lyophilized human plasma samples containing 17-OHP with the certified values determined by recognized RMPs was made. Moreover, the cRMP was successfully applied to measure 17-OHP in plasma samples and assessed the accuracy of an immunoassay in clinical laboratory.

RESULTS AND DISCUSSION

1. Analysis of 17-OHP by ID-LC-MS/MS



2. Method optimization

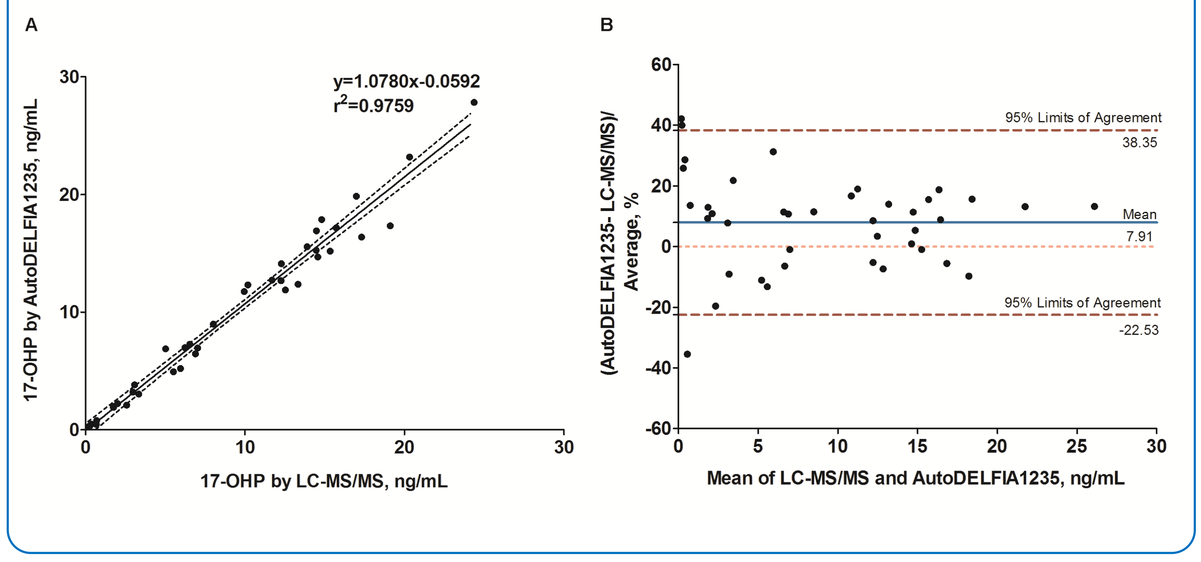


In order to get accurate results and achieve a maximum response (peak area) and a minimum base-line noise, along with a short run time, several parameters affecting the chromatographic separation of the estrogens, such as the composition of mobile phase, the nature and concentration of buffer and the concentration of the buffer were evaluated. 1. 17-OHP, 2.0Desoxycorticosterone, 3. Aldosterone, 4. Cortisol, 5. Corticosterone, 6. 11-Deoxycortisol, 7. Androstenedione, 8. DHEA, 9. Testosterone, 10. Pregnenolone, 11. 17α-Hydroxypregnenolone, 12. Stanolone, 13. Progesterone

3. Analytical characteristics

Analytical characteristics	17-OHP			
3.1 Precision				
Concentration, ng/mL	0.83	15.19	64.22	313.46
Intra-assay (n=15, %)	2.43	1.78	0.71	0.70
Inter-assay(n=15×3, %)	3.22	2.35	1.89	1.16
3.2 Linearity				
Linear range, ng/mL	0.47-958.63			
linear response	y=1.00334x-0.0150			
r^2	0.9992			
3.3 LODs and LOQs				
LOD, pg/mL (CV, n=4)	2.1			
LOQ, pg/mL (CV, n=4)	4.6			
3.4 Accuracy				
Concentration, ng/mL	0.40	20.00	150.00	
Average recovery(n=4) %	100.58	98.05	102.24	

4. Method comparison



The cRMP used for the 17-OHP measurements of the plasma samples of 40 patients was compared with AutoDELFIA1235 immunoassay analyzer. The sample concentrations were span from 0.15 ng/mL to 24.37 ng/mL.In hence, all of the samples were stable in the research period. Linear regression and Bland-Altman plots were used to evaluate the results.The respective coefficient of determination, r^2, of the immunoassay obtained from the linear regression equation provided by GraphPad Prism 5 software were as follows: r^2 = 0.9759 (AutoDELFIA1235 = 1.0780 LC-MS/MS–0.0592, 95% confidence interval (CI) for the slope 1.0220–1.1330, 95% CI for the intercept: -0.6707–0.5522 pg/mL, $S_{y|x}$ = 1.1310, P < 0.0001).

CONCLUSIONS

1. A highly reliable, accurate, and precise ID-LC-MS/MS-based candidate reference measurement procedure for 17-OHP measurement was developed and validated.
2. Extraction efficiency of sample preparation and sensitivity are better than previous researches, and appropriate retention of 17-OHP and high resolution of structural analogs were achieved within a short time span.
3. The results were in good agreement with the results of established reference measurement procedures.
4. The validated method was successfully applied to measure the 17-OHP level in serum samples and to assess the accuracy of an immunoassay in a clinical laboratory.

ACKNOWLEDGMENTS

We gratefully appreciate the financial support the National Key Research and Development Program of China (2017YFF0205401), the National Natural Science Foundation of China (81572088), the Natural Science Foundation of Guangdong Province (2018A0303130124), the Specific Research Fund for TCM Science and Technology of Guangdong Provincial Hospital of Chinese Medicine (YN2016QJ15), Guangzhou science and technology project (201704020213).