

Development of a TurboFlow LC-MS/MS Method for Quantitation of 17-Hydroxyprogesterone In Human Serum

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Introduction

17-hydroxyprogesterone (17-OHP) is a steroid hormone precursor whose quantitation is used for the research of congenital adrenal hyperplasia (CAH). 17-OHP is traditionally quantified by immunoassays, however these methods are characterized by a high rate of bias towards higher quantitative numbers from antibody cross-reactivity with structurally similar steroids. The use of more selective techniques like liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) is recommended (1,2) for quantitation of individual steroid compounds. We hereby report the results obtained by Thermo Scientific™ TurboFlow™ online sample extraction combined with tandem mass spectrometry with a comparison the immunoassay approach.

Methods

Sample Preparation

64 donor serum samples obtained from clinical research analysis (already quantified by RIA) were analyzed. 100 μ L of serum sample were protein precipitated by adding 100 μ L of internal standard solution (17-OHP- d_8 1 ng/mL in methanol). After centrifugation (5 minutes at 14000 rpm) the supernatant was directly injected onto a TurboFlow system equipped with Thermo Scientific™ Cyclone TurboFlow™ column, for further purification.

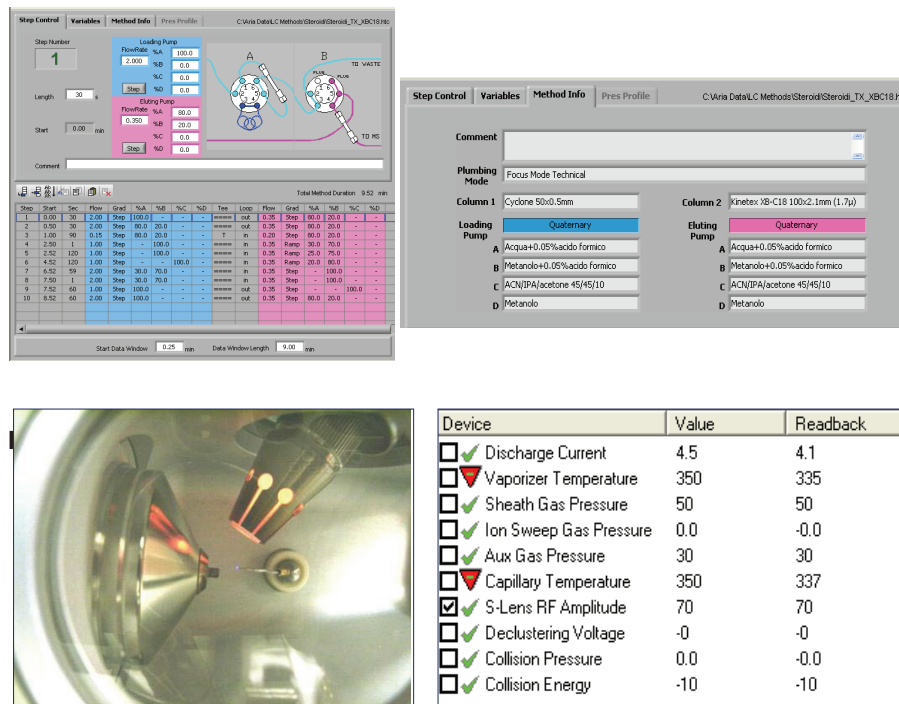
Liquid Chromatography

A TurboFlow Cyclone (50 x 0.5 mm) column was used as on-line extraction column. The chromatographic separation was obtained using a Kinetex C-18 XB (100 mm x 2.10 mm, (Phenomenex) column equilibrated with water and methanol containing 0.05% formic acid. The chromatographic parameters used in the method are shown in Figure 1.

Mass Spectrometry

17-OHP was analyzed using a Thermo Scientific™ TSQ Vantage™ mass spectrometer; acquisitions were performed by highly selected reaction monitoring (H-SRM) mode following the transitions of the precursor at m/z 331.3 and products at m/z 97.1 and 109.1 with collision energies of 23 and 28 eV, respectively. 17-OHP- d_8 was monitored following the transition at m/z 339.2 and product at m/z 100.2 with a collision energy of 28 eV. The source parameters are shown in Figure 2.

FIGURE 1: TurboFlow settings and chromatographic conditions



Data Analysis

Data acquisition and quantitative analysis were carried out using the Thermo Scientific™ Xcalibur™ 2.0.7 mass spectrometry software. Method comparison was performed with Bland-Altman plot and Passing Bablock regression analysis using Microsoft® Excel® 2010.

Results

The retention time of 17-OHP was about 5.9 min and no interferences were observed in the chromatographic run; a representative chromatogram is reported in Figure 3. The calibration curves were always linear over the concentration range of 0.02–10.0 ng/mL; the correlation coefficients (R^2) were higher than 0.99, which indicates excellent linear fit over the dynamic range; figure 4 shows a typical calibration curve. The assay was linear up to 50 ng/mL. Total imprecision (CV%) was lower than 5%; the lower limit of quantification was of 0.02 ng/mL (Table 1).

FIGURE 3: Typical SRM chromatogram of a serum sample

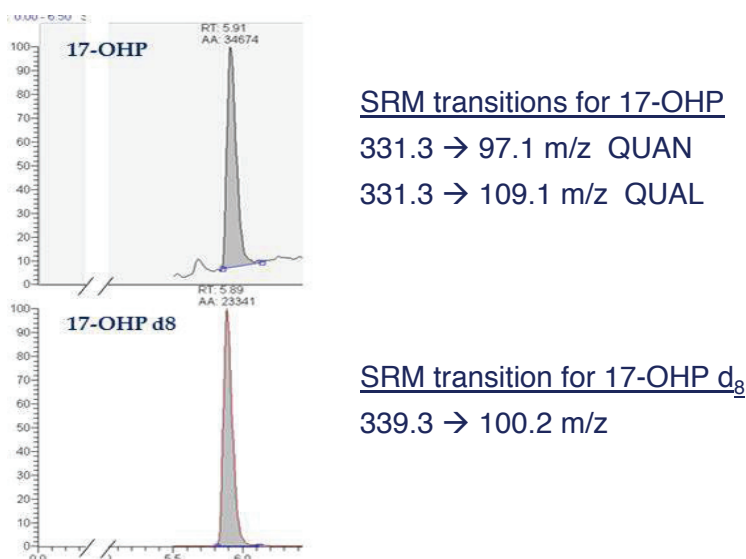


FIGURE 4: Representative seven point calibration curve for 17-OHP

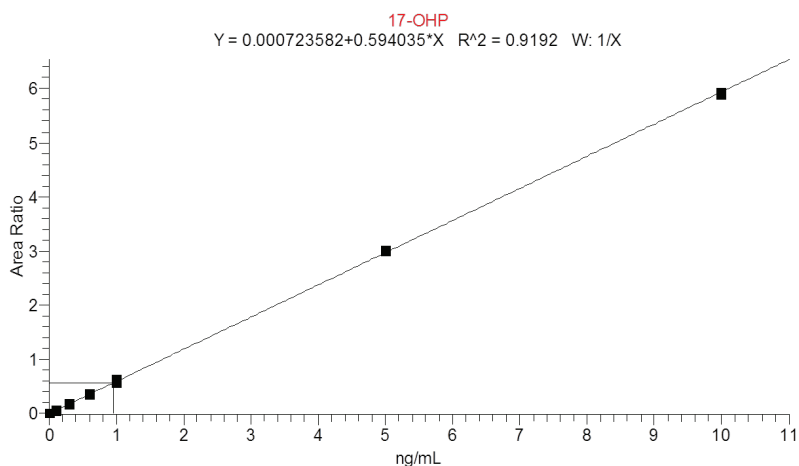


TABLE 1: Analytical validation data

LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)	Control levels (ng/mL)	Intra- assay CV (%)	Inter- assay CV (%)
0.01	0.02	50	0.3	2.49	7.82
			1.0	1.29	5.52
			2.0	1.26	9.29

These results were compared with the traditional immunoassay approach for the same analysis. Linear regression analysis shows an acceptable correlation between the two methods ($R^2: 0.892$) (Figure 5). 17-OHP values obtained with RIA method are higher than those obtained with LC-MS/MS, the mean difference being 0.67 ng/mL. Furthermore the Bland-Altman plot shows that these differences are directly dependent on analyte concentration (Figure 6).

FIGURE 5: Correlation between results obtained for 17-OHP using TurboFlow LC-MS/MS and immunoassay on 64 donor serum samples from clinical research

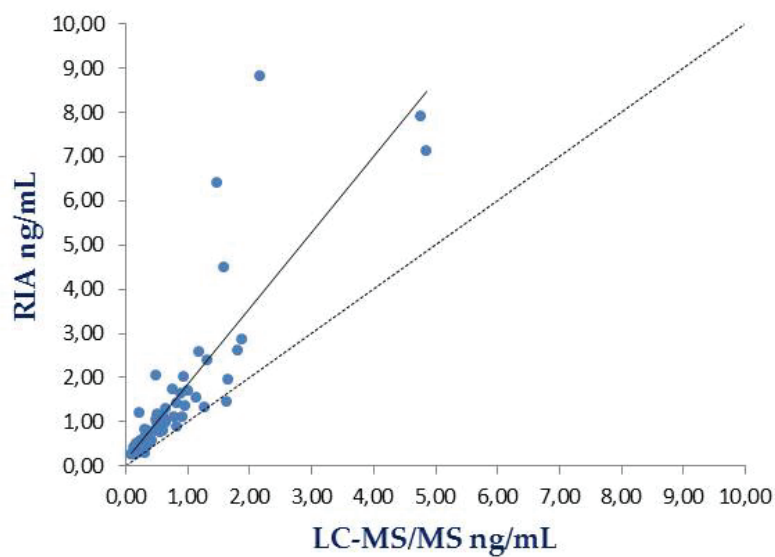
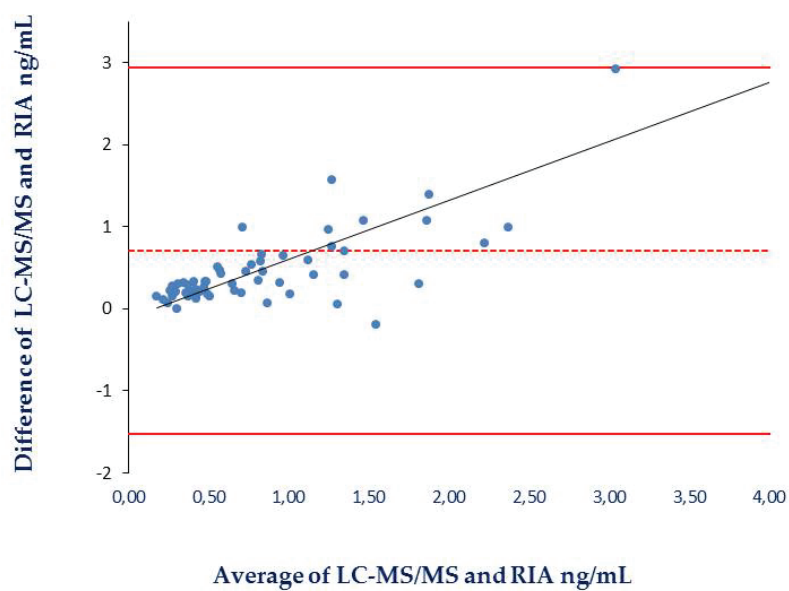


FIGURE 6: Bland-Altman plots of the differences between 17-OHP values obtained by TurboFlow LC-MS/MS and RIA methods



Conclusions

- TurboFlow online sample extraction provides a simple and effective clean-up procedure that minimizes interferences from the matrix, reduces human intervention to a minimum representing a cost- and time-effective approach.
- The TurboFlow LC-MS/MS method for the quantification of 17-OHP in serum samples for clinical research proved to be simple, analytically selective, precise and analytically sensitive.
- This method represents a valuable new approach that allows clinical research labs to effectively tackle the shortcomings of immunoassays.

References

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2. Fanelli F, Belluomo I, Di LV, et al. Steroids 2011;76:244–53.

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