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 µL of serum sample were protein precipitated by adding 100 µL 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.

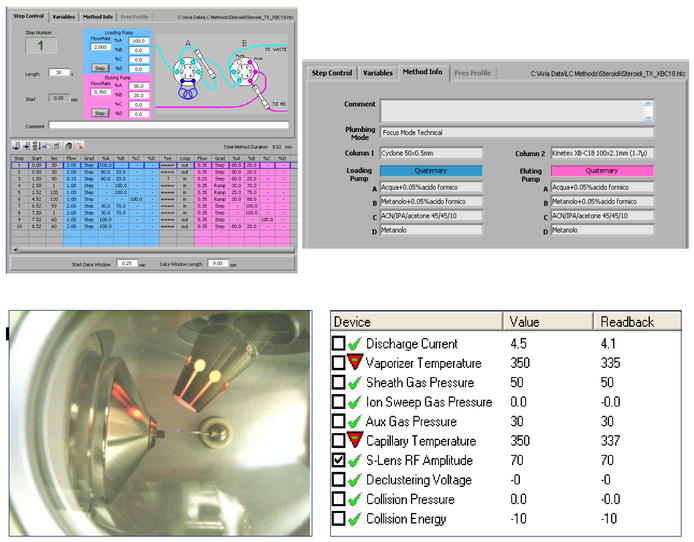
Liquid Chromatography

A TurboFlow Cyclone (50 x 0.5 mm) column was used as on-line extraction column. The chromatographic separation was obtained using a Kynetex 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



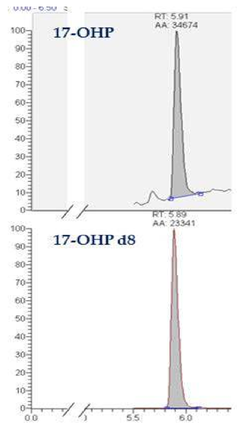
Data Analysis

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



SRM transitions for 17-OHP
331.3 → 97.1 m/z QUAN
331.3 → 109.1 m/z QUAL

SRM transition for 17-OHP d_8
339.3 → 100.2 m/z

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

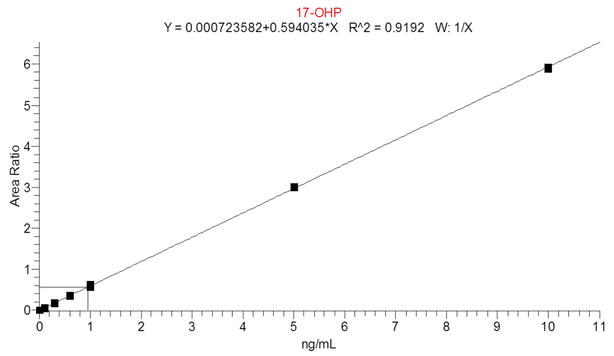


TABLE 1: Analytical validation data

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 (R2:0.892) (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

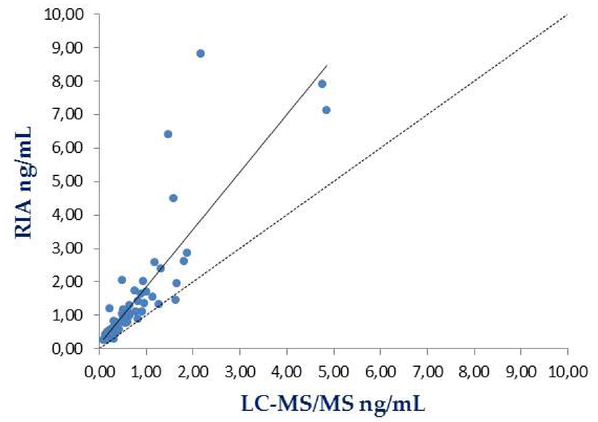
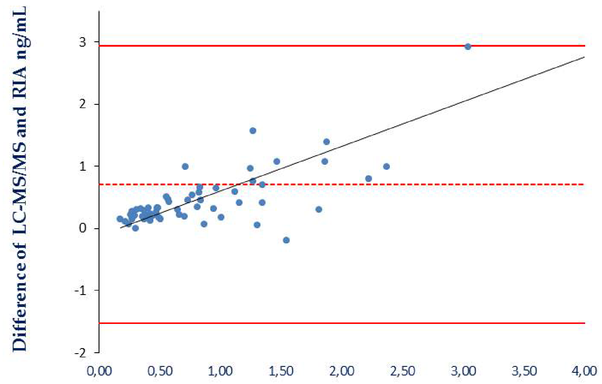


FIGURE 6: Bland-Altman plots of the differences between 17-OHP values obtained by TurboFlow LC-MS/MS and RIA methods



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

1. Wudy SA, Hartmann M, Svoboda M. Horm Res 2000; 53:68–71.
2. Fanelli F, Belluomo I, Di LV, et al. Steroids 2011;76:244–53.

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