Sensitive Quantitation of a Steroid Panel in Urine by LC-MS/MS using the AB SCIEX Triple Quad™ 6500 System

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INTRODUCTION

The measurement of steroids in urine can allow researchers to identify potential endocrine dysfunction in humans. Measurement of urinary steroid levels is useful in several areas of research, including evaluating risk of cancers of the reproductive system, serving as markers for and/or monitoring of adrenocortical tumors, and in the indication of stress disorders. The use of LC-MS/MS allows for highly sensitive and selective measurement of steroid panels in urine, which are necessary requirements for samples containing low levels of steroids and potentially high incidence of endogenous interferences.
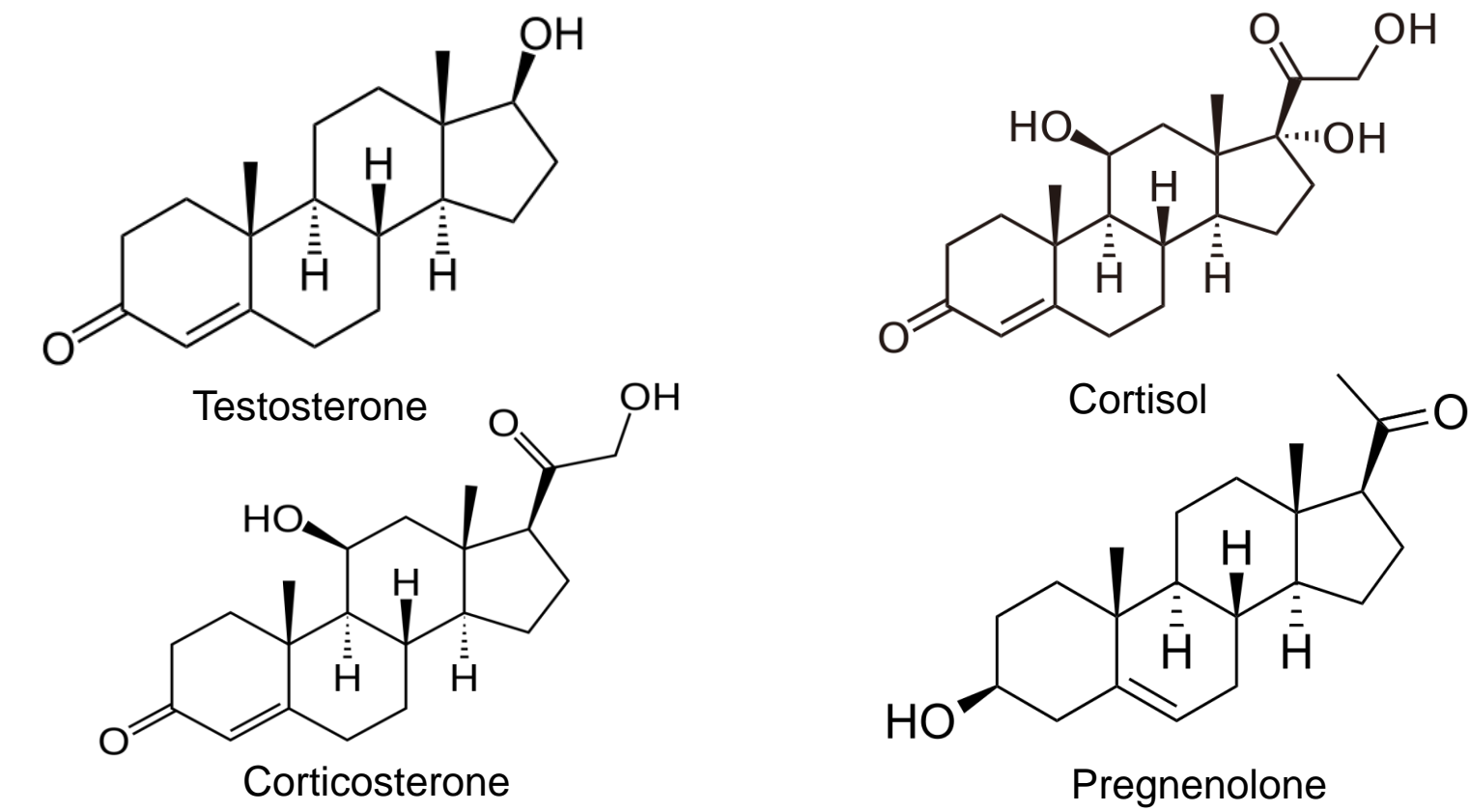

Figure 1. Representative steroids included in steroid panel.

Here we present a sensitive method for the analysis of a panel of steroids in electrospray positive ionization mode, using the AB SCIEX Triple Quad™ 6500 LC/MS/MS system. Calibration curves were prepared in synthetic urine over a large concentration range. Method performance was evaluated using these standards. The method was applied to several urine samples and preliminary results from that analysis are shown here.

MATERIALS AND METHODS

Sample Preparation:

Calibration standards were prepared in synthetic urine using stock solutions obtained from Cerilliant Corporation (Round Rock, Texas). Table 1 lists the analytes considered in the steroid panel along with calibration range and LOQ for each analyte. Internal standards were also used in each calibrator in order to correct for ionization variability.

Urine samples were prepared by adding 20 µL of a sodium acetate buffer (pH = 4.5) to 50 µL of urine. In addition, internal standard solution (25 µL) and 10 uL of β-glucuronidase were added to the urine. After vortex mixing, samples were heated to 55° C for 2 hours in order to enzymatically hydrolyze conjugated steroids. Samples were diluted, vortex mixed and centrifuged before being transferred to HPLC vials for injection onto the LC-MS/MS.

HPLC Conditions:

The analysis was done using a Shimadzu Prominence HPLC. Separation of the analytes was accomplished with a Phenomenex Kinetex C18 column (50x3.0, 2.6µ) at 0.5 mL/min. A 10 minute gradient allowed for the separation of all of the steroids. Figure 2 shows the critical separation of testosterone and DHEA and the separation of 11-DOC, 21-DOC and corticosterone. Mobile phases were water and methanol, both containing 0.1% formic acid. The injection volume used was 10 µL.
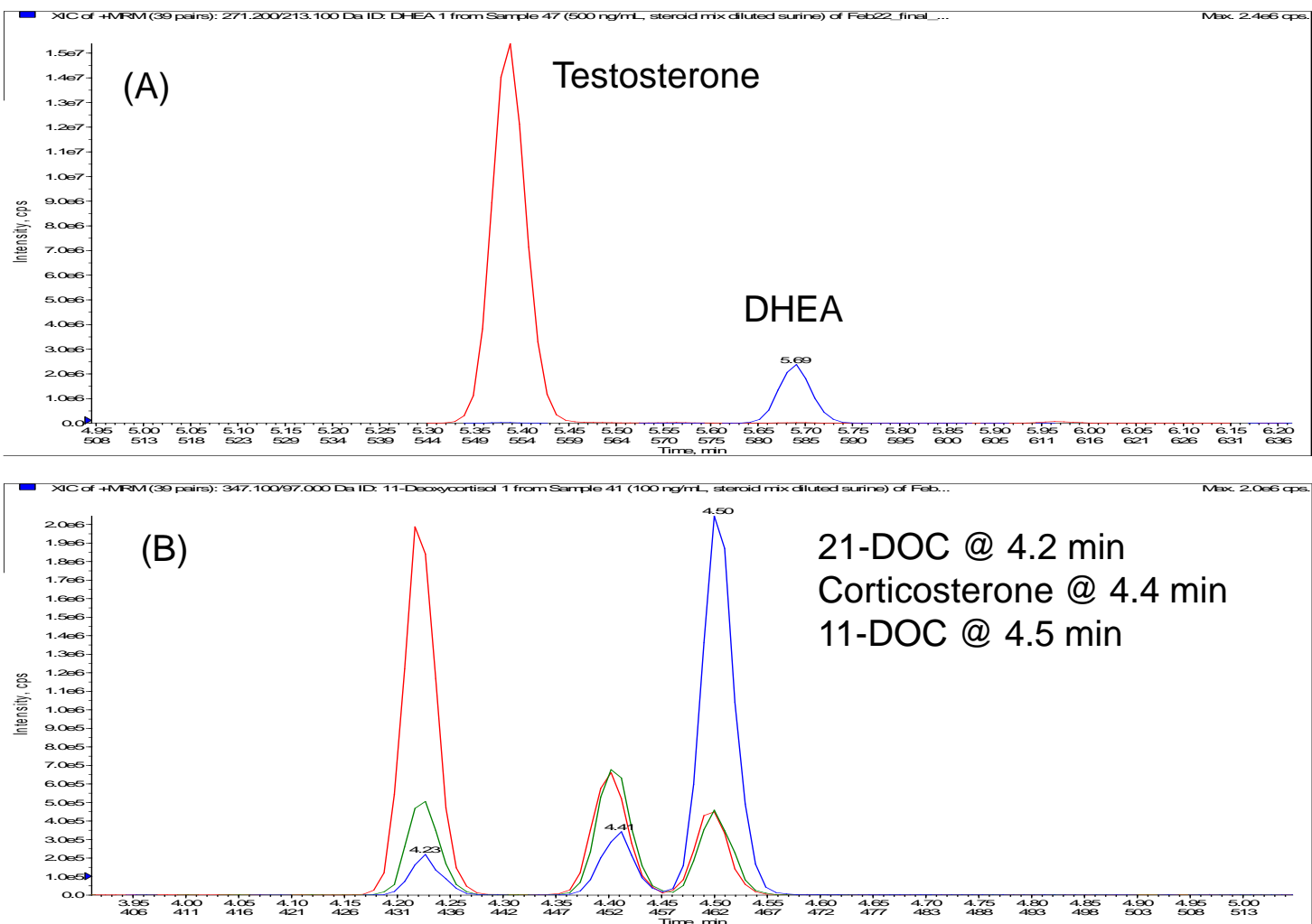

Figure 2. (A) Chromatogram showing separation of testosterone and DHEA (B) Chromatogram showing separation of 11-DOC, 21-DOC and corticosterone.

MS/MS Conditions:

MS/MS detection was accomplished using the AB SCIEX Triple Quad™ 6500 LC/MS/MS system equipped with IonDrive™ Turbo V source and operated in positive electrospray ionization mode. The Multiple Reaction Monitoring (MRM) mode was used, with 2 MRM transitions monitored per analyte.

RESULTS

The method presented here allowed quantification of the steroids listed in Table 1. Replicate injections (n=3) of the calibration standards demonstrated excellent linearity, accuracy and precision over the concentration range for each analyte. Accuracies ranged from 91 - 113% for all concentration levels for all analytes and linearity was >0.99 for all analytes. Measurement precision (%CV) was <20% for all analytes at all concentrations, and with the exception of calibrators at the LOQ for the analyte, %CV was generally much lower than 20%. The concentration range and LOQ achieved for each analyte is listed in Table 1.

Representative calibration curves and the LOQ (urine calibrator) chromatogram for testosterone and 17-hydroxyprogesterone are shown in Figure 3.

AB SCIEX Triple Quad™ 6500 LC/MS/MS System

Name	Calibration Range (ng/mL)	LOQ (ng/mL)
Testosterone	0.1 - 1000	0.1
17-hydroxyprogesterone (17-OHP)	0.1 - 1000	0.1
Cortisol	0.5 - 1000	0.5
Androstenedione	25 - 100	25
dehydroepiandrosterone (DHEA)	0.5 - 1000	0.5
dihydrotestosterone (DHT)	0.25 - 1000	0.25
11-deoxycortisol (11-DOC)	0.1 - 1000	0.1
21-deoxycortisol (21-DOC)	0.5 - 1000	0.5
Pregnenolone	1 - 1000	1
Dehydroepiandrosterone sulfate (DHEAS)	10 - 1000	10
Corticosterone	0.25 - 1000	0.25

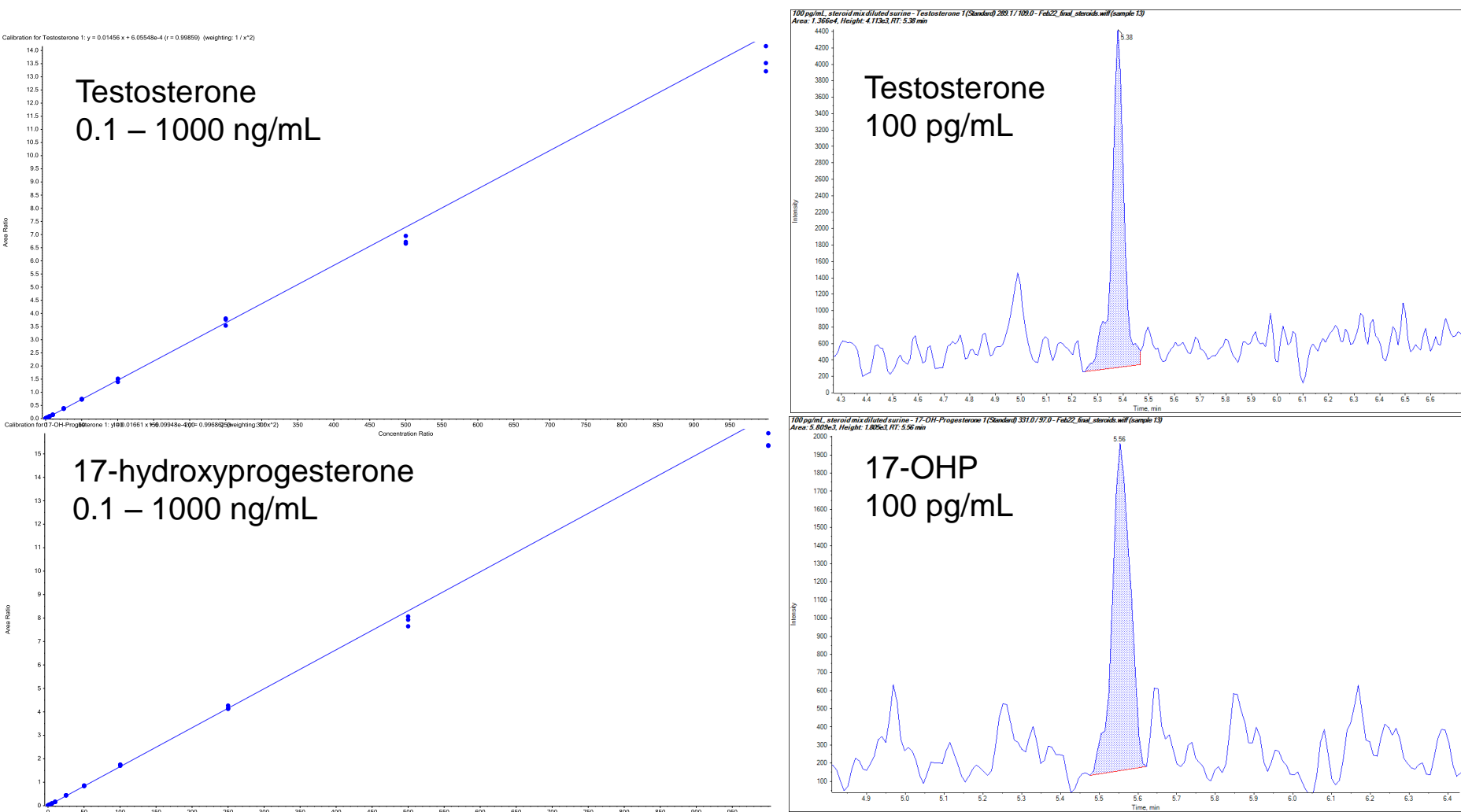*Table 1. Summary of steroid calibration concentration ranges and LOQ achieved using method.*

Figure 3. Calibration curves for Testosterone (top) and 17-hydroxyprogesterone (bottom). A chromatogram for each steroid at the method LOQ is also shown.

To evaluate the multi-steroid method, several urine samples were analyzed. Shown below in Figure 4 are representative chromatograms for several analytes detected and quantified in the urine samples. These preliminary results suggest that the method will be useful in quantifying steroids in urine at the required levels.
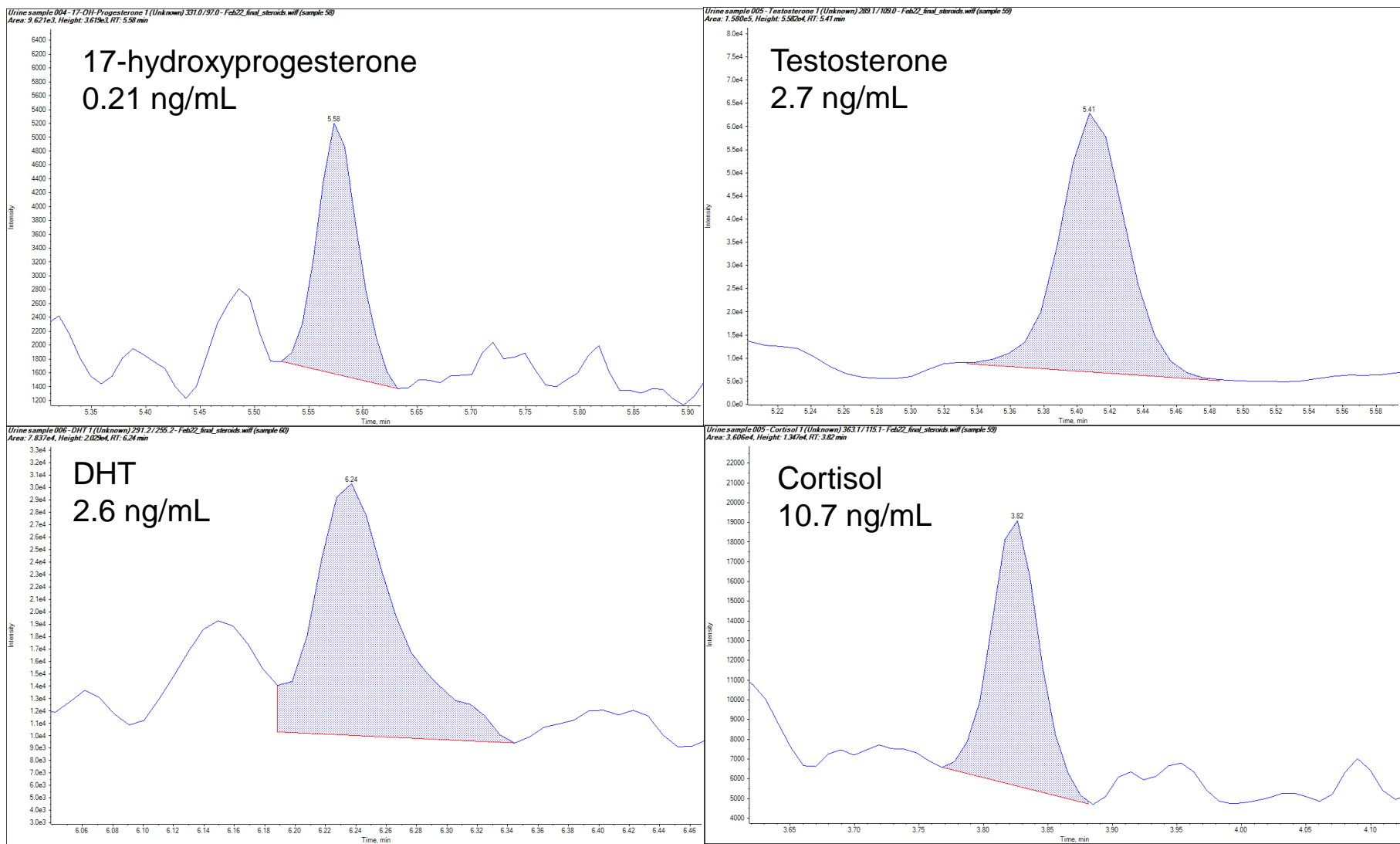

Figure 4. Chromatograms showing several steroids that were detected and quantified in urine samples.

CONCLUSIONS

We have developed a multi-steroid method for the detection of various steroids of interest in urine. The limits of quantitation achieved allow quantitation of steroids at the relevant urinary concentration ranges. Expansion of the panel to include other steroids is possible, therefore increasing the utility of the method for steroid research.

TRADEMARKS/LICENSING