Technical note | 003505

Clinical research

Quantification of steroids in human serum by TurboFlow online sample cleanup using a Transcend VTLX-1 UHPLC system

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Keywords

Transcend VTLX-1 system, TurboFlow technology, online sample cleanup, steroids, serum, LC-MS/MS, TSQ Altis MS

Application benefits

- Utilization of a new, compact, and cost-effective fully Vanquish-based Transcend UHPLC system for rapid online sample cleanup
- Simplified pre-injection sample preparation
- Quantification of eight steroids in five minutes
- Improved chromatographic performance with optimized Vanquish fluidics

Goal

To implement an analytical method for quantifying eight steroids in human serum using Thermo Scientific™ TurboFlow™ online sample cleanup on the new Thermo Scientific™ Transcend™ VTLX-1 UHPLC system, coupled to a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer. The study aims to compare the analytical performance with the same method run on a Thermo Scientific™ Transcend™ TLX-1 UHPLC system in terms of chromatography, limits of quantification, accuracy, and intra-assay precision.

Introduction

Liquid chromatography (LC) coupled with triple quadrupole tandem mass spectrometry (MS/MS) has become the preferred technology for quantifying steroid hormones in biological matrices. Its superior specificity compared to immunoassays and its sensitivity when using low sample volumes have been major contributors to its success. However, as LC-MS/MS technology continues to proliferate, laboratory space and budget constraints often pose significant hurdles to adoption.

To address these needs, we have evolved our Transcend TLX-1 UHPLC system into the new Transcend VTLX-1 UHPLC system, which has a smaller footprint while maintaining the same functionalities as its predecessor (Figure 1). The Transcend VTLX-1 system can perform TurboFlow online sample cleanup or online solid phase extraction (SPE) (Figure 2a), as well as UHPLC applications that do not require online purification (Figure 2b). Additionally, replacing the Thermo Scientific™ TriPlus™ RSI autosampler with a stackable Thermo Scientific™ Vanquish™ Dual Split sampler dramatically reduces the system's footprint, alleviating space constraints in the laboratory.

In this technical note, we analyzed the same samples on both a Transcend VTLX-1 system and a Transcend TLX-1 system, alternately coupled to the same TSQ Altis triple quadrupole mass spectrometer. Serum samples were extracted by offline protein precipitation with concurrent internal standard addition. Extracted samples were injected onto both Transcend UHPLC systems. Detection was performed in Selected Reaction Monitoring (SRM) acquisition mode using a TSQ Altis mass spectrometer with a heated electrospray ionization (HESI-II) source operated in positive ionization mode. Method performance was evaluated using calibrators, controls, and internal standards from the ClinMass™ Complete Kit for Steroids in Serum/Plasma from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of chromatography, linearity of response within the calibration ranges, accuracy, and intra-assay precision for each analyte.

Figure 1. (a) Transcend TLX-1 and (b) Transcend VTLX-1 systems

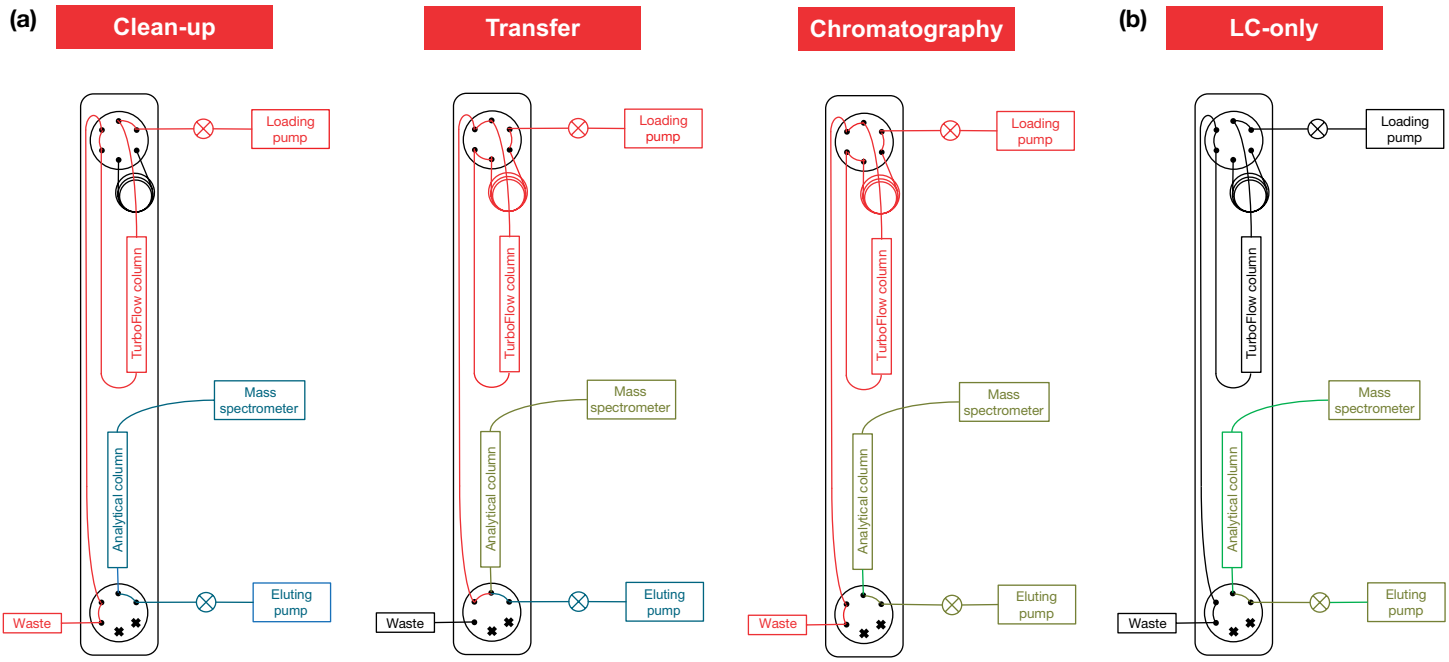

Figure 2. Schematic representations of a Transcend VTLX-1 UHPLC system operated in TurboFlow mode (a) and operated in LC-only mode (b)

Experimental

Target analytes

Target analytes, corresponding internal standards, and concentration ranges covered by the calibrators used are reported in Table 1.

Sample preparation

Lyophilized calibrators (MS12013 lot #2214) and controls (MS12083 lot #2214) were redissolved in water following the indications from RECIPE to the final nominal concentrations reported in Table 2 and Table 3, respectively.

Redissolved calibrators and controls were divided into 200 µL aliquots and stored at -80 °C waiting for extraction. Calibrators (in replicates of two) and controls (in replicate of six) were thawed at room temperature and protein precipitated using 200 µL of methanol containing six isotopically labelled internal standards for quantification. Precipitated samples were left at room temperature for 5 minutes, vortex-mixed again, left at 4 °C for an additional 15 minutes, and centrifuged at 10 °C for 10 minutes at maximum speed. The supernatant was transferred to clean vials and preserved at 10 °C in the autosampler pending the injection.

Table 1. Target analytes, internal standards, and concentration ranges covered by calibrators

Analyte	Internal standard	Concentration range (µg/L)
Androstenedione	d7-Androstenedione	0.120–15.4
Cortisol	d4-Cortisol	2.37–295
11-Deoxycortisol	d5-11-Deoxycortisol	0.109–15.1
21-Deoxycortisol	d5-11-Deoxycortisol	0.109–14.2
17-Hydroxyprogesterone	d8-17-Hydroxyprogesterone	0.130–14.4
21-Hydroxyprogesterone	d8-17-Hydroxyprogesterone	0.187–26.4
Testosterone	d3-Testosterone	0.101–10.8
DHEAS	d5-DHEAS	40.3–5240

Table 2. Nominal concentrations (µg/L) for calibrators (MS12013 lot #2214)

Analyte	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6
Androstenedione	0.120	0.453	0.935	1.91	7.61	15.4
Cortisol	2.37	9.50	19.2	38.6	151	295
11-Deoxycortisol	0.109	0.458	0.937	1.92	7.56	15.1
21-Deoxycortisol	0.109	0.423	0.845	1.70	6.99	14.2
17-Hydroxyprogesterone	0.130	0.462	0.922	1.79	7.24	14.4
21-Hydroxyprogesterone	0.187	0.826	1.65	3.33	13.5	26.4
Testosterone	0.101	0.349	0.705	1.35	5.47	10.8
DHEAS	40.3	157	324	653	2573	5240

Table 3. Nominal concentrations (µg/L) for controls (MS12083 lot #2214)

Analyte	CTRL 1	CTRL 2	CTRL 3
Androstenedione	0.241	0.680	3.83
Cortisol	4.80	13.7	77.0
11-Deoxycortisol	0.228	0.672	3.83
21-Deoxycortisol	0.222	0.628	3.55
17-Hydroxyprogesterone	0.242	0.659	3.71
21-Hydroxyprogesterone	0.414	1.18	6.70
Testosterone	0.186	0.504	2.74
DHEAS	79.2	230	1309

Liquid chromatography

A Transcend VTLX-1 UHPLC system (P/N 60500-60301) and a Transcend TLX-1 UHPLC system (P/N 60500-60201) were coupled on two different days to a TSQ Altis triple quadrupole mass spectrometer to evaluate the chromatographic performance of the two front ends. The same analytical method, including TurboFlow online sample cleanup, was used on the two systems (Figure 3). Sample cleanup was performed using Fisher Chemical™ Optima™ LC/MS water (P/N W6-1) and methanol (P/N A456-1) on a Thermo Scientific™ Cyclone-P 0.5 × 50 mm TurboFlow column (P/N CH-953289). Chromatographic separation was achieved on a Thermo Scientific™ Accucore™ Biphenyl 50 × 2.1 mm, 2.6 µm column (P/N 17826-052130) using Optima LC/MS water and methanol with 0.2 mM ammonium fluoride (P/N 393190500). Total runtime was 5.0 minutes.

Mass spectrometry

Analytes and internal standards were detected in SRM acquisition mode on a TSQ Altis mass spectrometer with heated electrospray ionization operated in positive ion mode. A summary of the MS conditions is reported in Table 4. A comprehensive list of SRM parameters used to acquire analytes and internal standards are reported in Table 5.

Table 4. MS settings

Ion source parameters	
Source type	HESI-II
Spray voltage - Positive (V)	3,000
Sheath gas (Arb)	65
Aux gas (Arb)	20
Sweep gas (arb)	0
Ion transfer tube temp. (°C)	350
Vaporizer temp. (°C)	400
S-Lens RF level	60
Settings	
Data acquisition mode	SRM
SRM parameters	
Cycle time (s)	0.3
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	1.2
Chromatographic peak width (s)	6

Performance comparison

The performance of the two different front ends when running the same TurboFlow method was compared in terms chromatographic peak symmetry, linearity of response within the calibration ranges, accuracy, and intra-assay precision for all the analytes on a single acquisition batch.

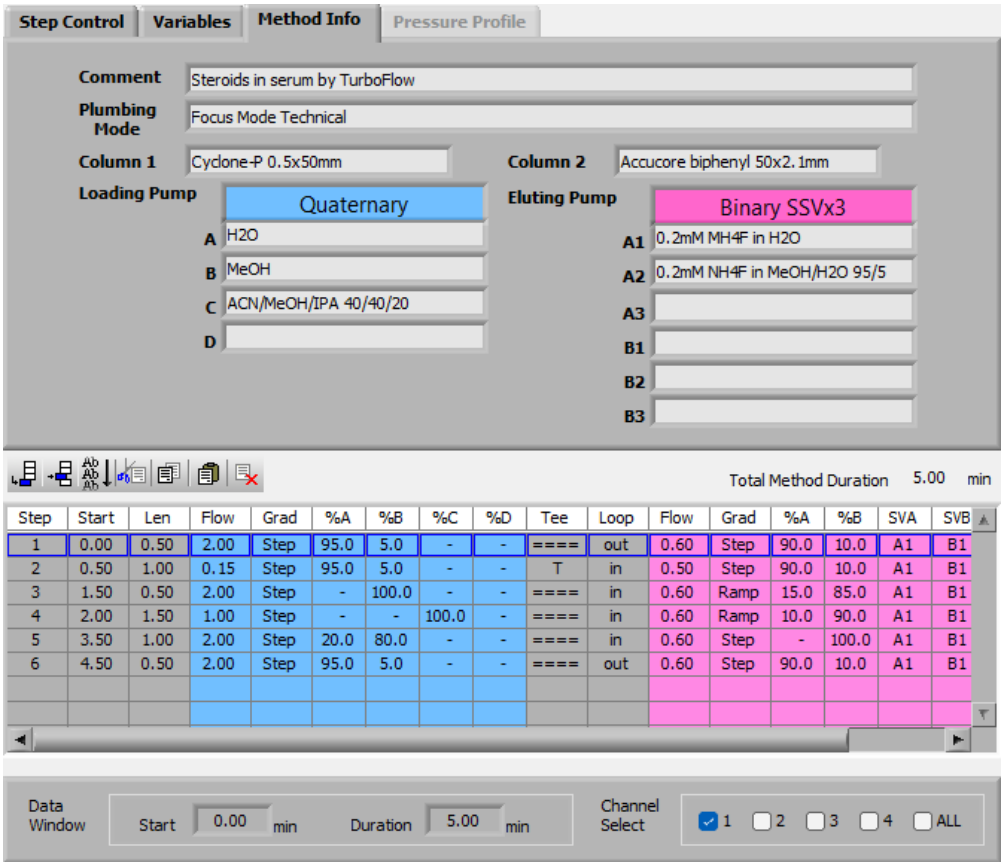

Figure 3. LC method description including both TurboFlow sample cleanup (blue) and chromatographic separation (pink) details

Table 5. SRM parameters

Compound	RF Lens	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)
Androstenedione	54	287.05	96.90	23
Cortisol	52	363.05	120.90	26
11-Deoxycortisol	35	347.10	96.90	26
21-Deoxycortisol	47	347.10	311.00	16
17-Hydroxyprogesterone	50	331.10	96.90	26
21-Hydroxyprogesterone	51	331.10	96.90	24
Testosterone	50	289.05	96.90	24
DHEAS	51	271.05	213.00	16
d7-Androstenedione	54	294.15	99.90	23
d4-Cortisol	52	367.05	120.90	26
d5-11-Deoxycortisol	35	352.10	99.90	26
d8-17-Hydroxyprogesterone	50	339.10	99.90	26
d3-Testosterone	50	292.05	96.90	24
d5-DHEAS	51	276.05	218.00	16

Chromatographic peak performance was evaluated by comparing peak symmetry and sharpness for each analyte using the highest calibrator.

Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using the quality control samples at three different levels provided by RECIPE prepared and analyzed in replicates of six (n=6).

Intra-assay precision was evaluated in terms of percentage coefficient of variation (%CV) using the controls at three different levels in replicates of six (n=6).

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 5.1 software.

Results and discussion

Comparison of peak symmetry and intensity obtained on both front ends for the highest calibrator showed a superior peak symmetry for all analytes using the Transcend VTLX-1 system, thanks to the optimized fluidics (Figure 4). In the case of dehydroepiandrosterone (DHEAS), the analyte showed no chromatographic peak when using the Transcend TLX-1 system; on the contrary, the same analytical method generated an extremely symmetric peak. This means easier TurboFlow method development as well as a considerably higher signal intensity (except for androstenedione and 21-hydroxyprogesterone, which showed similar peak shape and intensity).

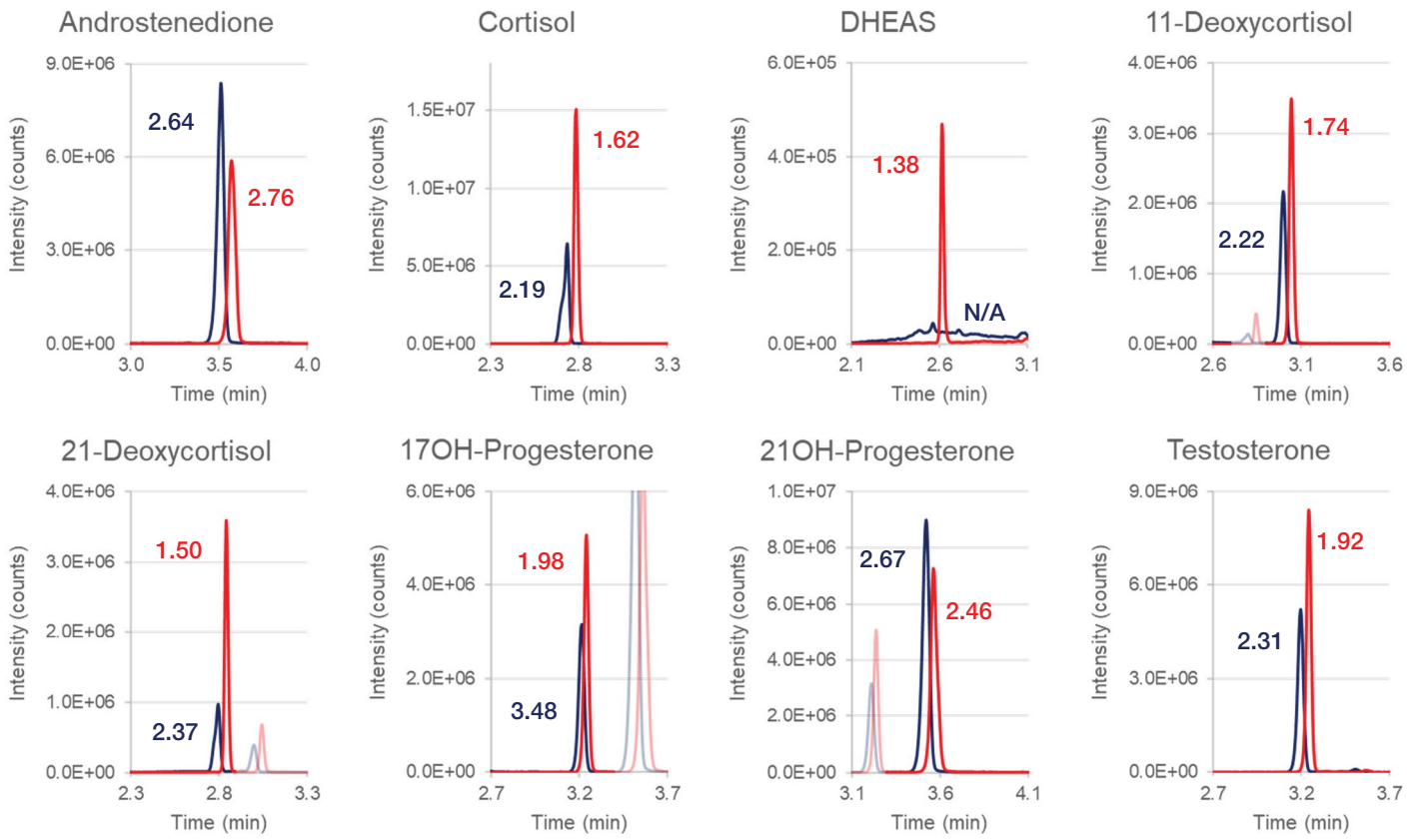

Figure 4. Chromatographic peak performance comparison (— Transcend TLX-1 system, — Transcend VTLX-1 system) including full width at half maximum (FWHM) comparison (in seconds)

A linear response with 1/x weighting was obtained for all the analytes on both systems, with an excellent minimum correlation factor (R^2) of 0.9995 and 0.9996 for the Transcend TLX-1 system and Transcend VTLX-1 system, respectively. Representative chromatograms of the lowest calibrator versus the blank for testosterone and 17-hydroxyprogesterone on both Transcend systems are reported in Figure 5. Representative calibration curves in the concentration range covered by the calibrators from RECIPE for the same analytes are shown in Figure 6.

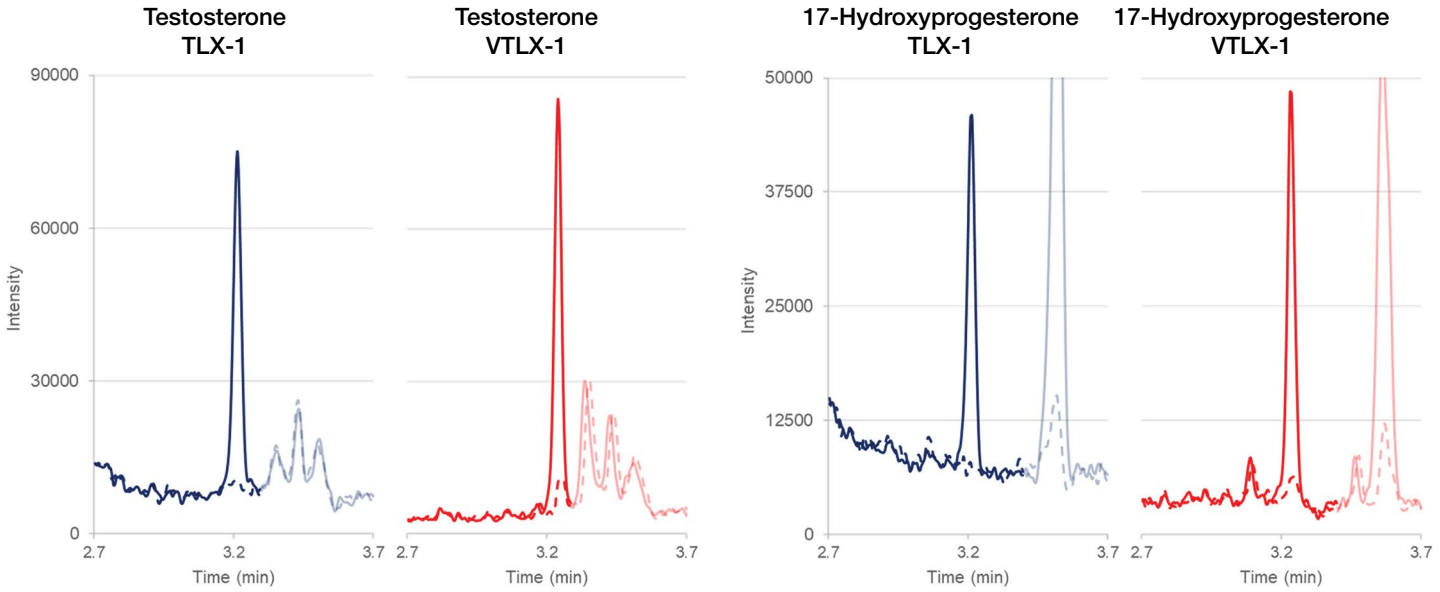

Figure 5. Representative chromatograms of the lowest calibrator for testosterone and 17-hydroxyprogesterone. — CAL1 Transcend TLX-1 system, — CAL1 Transcend VTLX-1 system, --- CAL0 Transcend TLX-1 system, --- CAL0 Transcend VTLX-1) system.

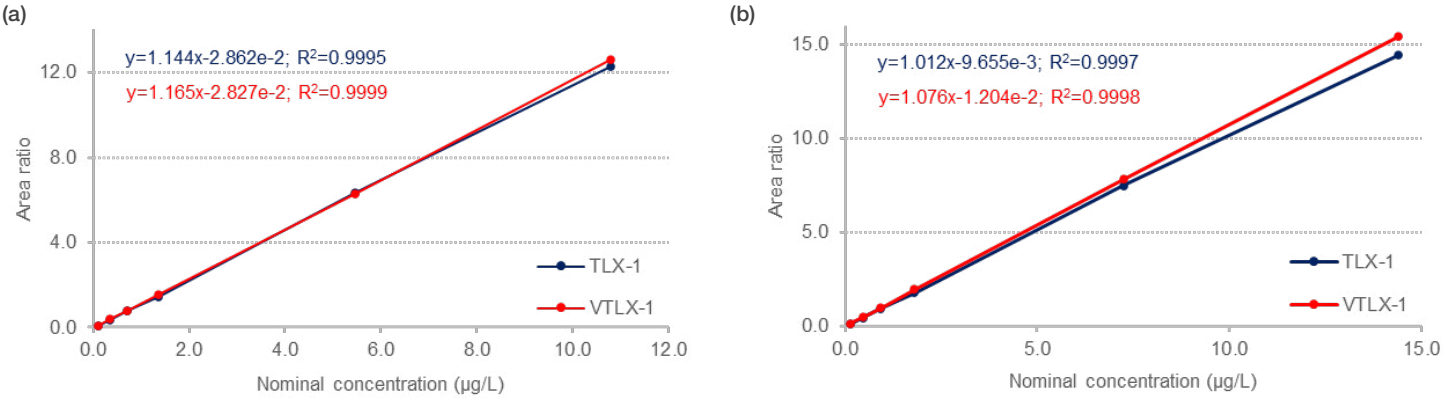

Figure 6. Representative calibration curves for (a) testosterone and (b) 17-hydroxyprogesterone

Experimental data demonstrates the outstanding equivalent performance of this method when applied to the two different Transcend systems, both in terms of accuracy and intra-assay precision. A graphical comparison between nominal and average (n=6) measured concentration for the three control levels for each analyte is reported in Figure 7, showing an excellent correlation between the use of the two different Transcend systems. The percentage bias between nominal and back-calculated concentration was always within ±5.6% and within -6.2% and 4.5% for all calibrators on the Transcend TLX-1 and Transcend VTLX-1 systems, respectively. The %CV for intra-assay precision was always below 2.3% for both systems (Table 6).

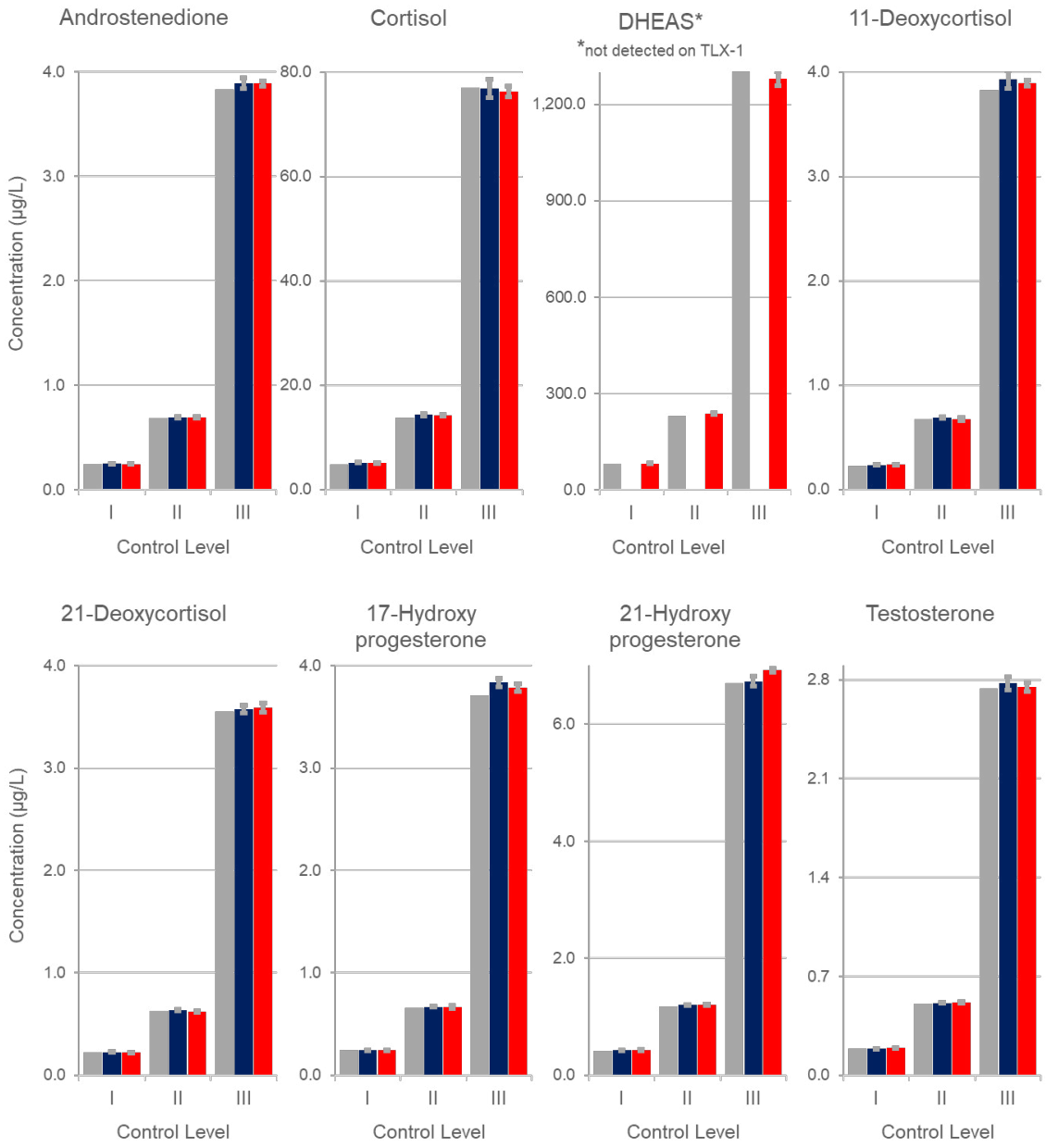

Figure 7. Comparison between nominal and average measured concentration and standard deviation (n=6) for control samples Level I, II and III (Nominal, Transcend TLX-1 system, Transcend VTLX-1 system)

Table 6. Analytical accuracy (%Bias) and intra-assay precision (%CV) results for controls MS12083 lot #2214

Analyte	Level	Nominal concentration (ng/L)	Transcend TLX-1 system			Transcend VTLX-1 system		
			Average calculated concentration (ng/L)	Bias (%)	CV (%)	Average calculated concentration (ng/L)	Bias (%)	CV (%)
Androstenedione	I	0.241	0.248	2.9	1.1	0.243	0.6	1.7
	II	0.680	0.690	1.4	1.1	0.691	1.6	0.7
	III	3.83	3.89	1.5	1.3	3.89	1.5	0.6
Cortisol	I	4.80	5.12	6.7	1.6	5.06	5.3	0.5
	II	13.7	14.3	4.3	1.9	14.2	4.0	1.1
	III	77.0	76.9	-0.2	2.3	76.3	-1.0	1.4
DHEAS	I	79.2	N/A	N/A	N/A	80.9	2.1	1.6
	II	230	N/A	N/A	N/A	235	2.6	0.9
	III	1309	N/A	N/A	N/A	1278	-2.3	1.8
11-Deoxycortisol	I	0.228	0.235	3.1	1.0	0.238	4.2	0.8
	II	0.672	0.688	2.3	0.9	0.670	-0.2	2.3
	III	3.83	3.92	2.6	2.2	3.89	1.7	0.8
21-Deoxycortisol	I	0.222	0.225	1.5	0.8	0.221	-0.5	1.6
	II	0.628	0.632	0.7	1.1	0.622	-1.0	1.2
	III	3.55	3.57	0.6	1.0	3.59	1.2	1.2
17-Hydroxyprogesterone	I	0.242	0.243	0.5	1.5	0.242	-0.2	1.0
	II	0.659	0.669	1.5	1.0	0.668	1.3	2.2
	III	3.71	3.84	3.4	1.0	3.79	2.0	1.1
21-Hydroxyprogesterone	I	0.414	0.423	2.2	1.0	0.426	2.9	2.2
	II	1.18	1.20	1.9	0.5	1.20	2.0	1.3
	III	6.70	6.73	0.5	1.2	6.92	3.3	0.5
Testosterone	I	0.186	0.190	2.1	0.6	0.192	3.4	0.4
	II	0.504	0.510	1.2	0.7	0.513	1.8	1.3
	III	2.74	2.78	1.3	1.6	2.751	0.4	1.2

Conclusions

An analytical performance comparison between the new Transcend VTLX-1 UHPLC system and the Transcend TLX-1 version was performed using the quantification of a panel of eight steroids in human serum as a reference application. The two Transcend systems were alternatively coupled to the same TSQ Altis triple quadrupole mass spectrometer for detection. Calibrators, controls, and internal standards from RECIPE were used on a home-made TurboFlow method; the total runtime was 5.0 minutes. The method incorporates simple offline protein precipitation with concurrent addition of the internal standards.

It was not possible to obtain acceptable chromatography for DHEAS using the Transcend TLX-1 system, where the considerable delay volume due to long stainless-steel tubing would have required extensive method optimization.

For the remaining analytes, the new Transcend VTLX-1 system showed significantly improved chromatographic peak sharpness and symmetry, especially for the early eluting compounds, thanks to the optimized fluidics based on Thermo Scientific™ Viper™ fittings that reduce the delay volume to a minimum. This hardware enhancement also facilitates the development of analytical methods requiring online sample cleanup, minimizing analyte dispersion during the transfer from the extraction cartridge to the analytical column.

The two Transcend systems also demonstrated equivalent analytical performance in terms of linearity of response within the covered calibration range, with overlapping linear calibration curves for each analyte (except for DHEAS on the Transcend TLX-1 system) and correlation factors always above 0.9995. Similar excellent performance was also obtained in terms of accuracy and intra-assay precision, with a percentage bias within ±6.7% and a percentage CV always below 2.3%.

The obtained results demonstrate the ability of the new Transcend VTLX-1 UHPLC system to provide higher quality analytical results when compared to the Transcend TLX-1 version in the quantification of steroids in human serum, meeting research laboratory requirements in terms of linearity of response, accuracy, and precision.

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