Supelco®
Analytical Products

Application Note

Steroid Hormone Analysis in Serum using Supel™ Swift HLB DPX Tips

Introduction

Routine hormone analysis is necessary for establishing and monitoring patient diseases. For example, the continuous monitoring of cortisol levels can help diagnose a patient with Cushing disease (high cortisol) or Addison disease (low cortisol). A robust method for steroid hormone determination in serum is imperative in diagnostics and treatment. The Supel™ Swift HLB DPX Tips (3 mg bed, Hamilton®) allow for reduced sample volume, sample evaporation mitigation, and offer a fully automated approach. In this method, a total of 9 steroids (cortisone, cortisol, 11-deoxycortisol, androstenedione, testosterone, dehydroepiandrosterone, 5α-dihydrotestosterone, 17α-hydroxyprogesterone, and progesterone) were analyzed as a panel to provide a variety of testing applications and disease diagnostics.

Figure 1. Supel™ Swift DPX HLB 3 mg (bed) Tips. The tips are being actively picked up by the automated liquid handler.

The Supel™ Swift HLB sorbent provides good selectivity and sensitivity for steroids in a neutral solution[1], allowing for dilution with water prior to injection. The sorbent has significant versatility in analyte binding due to the co-polymer phase containing both hydrophilic and lipophilic functional groups.[1]

This validated method used 100 µL of serum and the final volume available for injection was approximately 100 µL, allowing for a 1:1 concentration factor without solvent evaporation. Recoveries for the 9 analytes range from 65-86% **(Table 7).** The LOQ's for all analytes fall below clinically relevant values, and possessed linear dynamic ranges between 0.025 ng/mL and 250 ng/mL. The automated extraction method allows up to 96 samples to be processed simultaneously in approximately 20 minutes prior to LC-MS/MS analysis.

Experimental

Methods

A Hamilton® Microlab NIMBUS96 was utilized to automate sample preparation using the Supel™ Swift DPX HLB Tips (**Figure 1**). The analysis was performed on an Agilent 1290 LC system coupled with a SCIEX Triple Quad™ 6500+ tandem mass spectrometer. The LC column used was an Ascentis® Express C18 (2.7 µm particle size, L × I.D. 10 cm × 3 mm) joined with an Ascentis® Express C18, 2.7 Micron Guard Cartridge in an Ascentis® Express Guard Cartridge Holder (Millipore Sigma, Burlington, MA); this combination allowed for optimal separation of all steroids. An injection volume of 15 µL was found to be optimal to meet required cutoffs. The LC conditions are shown in **Table 1.** Ammonium fluoride additives are common in steroid analysis but were found to decrease retention time stability and were therefore omitted. The mass spectrometer source parameters are available in **Table 2** with the transitions monitored in **Table 3.**

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Sample Preparation

Serum was aliquoted (100 µL) into a 2 mL V-bottom polypropylene well plate. The internal standard mixture (200 ng/mL for all internal standards except for DHT and progesterone which were 500 ng/mL) was added (10 µL) and allowed to incubate for 1 hour at ambient temperature. The well plate was then loaded onto the NIMBUS96 system for the rest of the automated protocol. The automated liquid handler, ALH, picked up a set of standard transfer tips, added 200 µL of aqueous 0.4% formic acid to the sample and mixed thoroughly. This solution was then incubated for 15 minutes prior to sample extraction. While the protein dissociation step occured, the ALH picked up a second set of transfer tips for aliquoting the wash solvents into appropriate well plates (**Figure 4**). After that the ALH picked up the Supel™ Swift HLB DPX Tips and conditioned the HLB sorbent by aspirating and dispensing 300 µL of 20% methanol from a buffer reservoir two times. Once the protein dissociation timer was complete, the ALH moved to the sample well plate and aspirated and dispensed the sample five times to bind analytes to the HLB sorbent. The ALH moved to the first wash location (300 µL of 100% water) and aspirated/dispensed three times, and sequentially moved to the second wash location (300 µL of 20% methanol) and aspirated/dispensed three times. The ALH ejected the Supel™ Swift HLB DPX Tips back into the original deck position and picked up the transfer tips to aliquot the elution solvent into the appropriate well plate. This was done to avoid solvent evaporation of the low elution volume while the previous steps of the method were completed. Finally, the ALH picked up the Supel™ Swift HLB DPX Tips again and moved to the elution location (75 µL 50/50 MeOH/ACN), aspirated/dispensed three times. The tips were ejected, and standard tips were picked up to dilute the eluent with 25 µL of water. The final sample plate was then sealed and vortexed briefly for 5-10 seconds before submitting for analysis by LC-MS/MS injection. **(Figures 2, 3,** & **4)**

100 µL Serum + 10 µL internal standards; incubate 1 hour

Add 200 µL of 0.4% formic acid; incubate 15 minutes

Condition, Bind, Wash, and Elute (see Figure 3)

Dilute with 25 µL water, ready for LC-MS analysis

Figure 2. Sample preparation method.

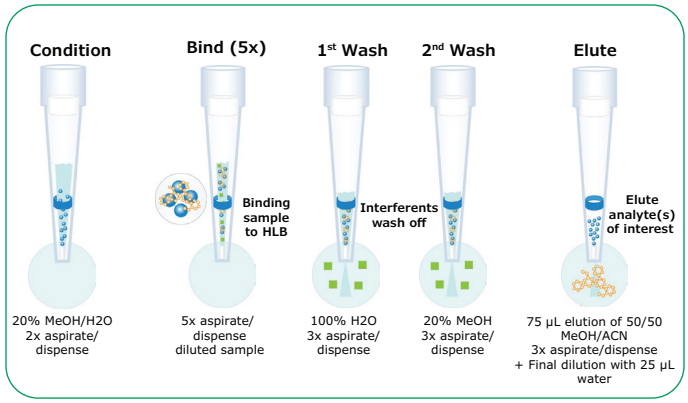


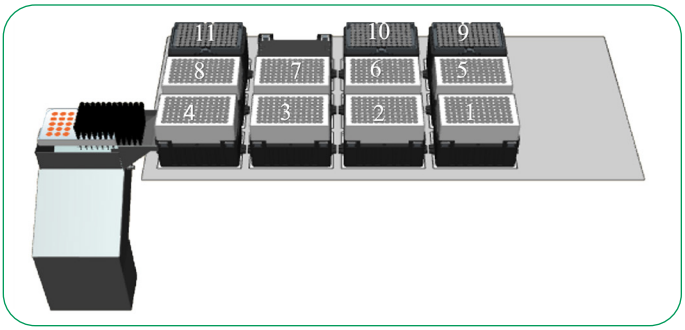
Figure 3. Schematic of the automated bind/wash/elute steps.

Ionization Effects and Recoveries

Ionization effects and recovery studies were performed as outlined by Scientific Working Group for Forensic Toxicology, SWGTOX.[5]

Briefly, recoveries were evaluated by preparing two sets of serum samples; the first that were spiked with internal standards prior to extraction, and the second set were serum samples that were spiked with internal standards after extraction.

Ion suppression/enhancement was evaluated by preparing two sets of samples. The first set being internal standards prepared in final solution composition (3 equivalents of 50/50 methanol/acetonitrile to 1 equivalent of water) and the second set being the internal standards spiked into the post-extracted solution. All internal standard concentration were as described earlier (200 ng/mL, except for DHT and progesterone which were 500 ng/mL).



#	Usage/Step Performed
1	**Sample plate** Preloaded manually with 100 µL serum + ALH adds 200 µL 0.4% formic acid (#5) in water, timer to wait 15 mins
2	**Wash Plate #1** + 300 µL water (from 7)
3	**Wash Plate #2** + 300 µL 20% methanol in water (from 6)
4	**Elution Plate** + 75 µL 50/50 methanol/acetonitrile (7) for elution + 25 µL water post-elution (6)
5	**0.4% Formic Acid in Water** • Used for protein dissociation of sample (1)
6	**20% Methanol in Water** • Used for loading wash plate #2 (3) and • Used for conditioning of tips
7	**Water** • Used for loading wash plate #1 (2)
8	**50/50 Acetonitrile and Methanol** • Used for elution in (4)
9	**Standard Transfer Tips (300 µL)** • One set used for the formic acid addition
10	**Supel™ Swift HLB DPX Tips, 3 mg (bed)**
11	**Standard Transfer Tips (300 µL)** • Used for the remaining transfer steps (adding elution solvent to the well plate, etc.)

Figure 4. Representative Hamilton® Microlab NIMBUS96 deck overview with designation of lab equipment and/or role in the method

Results & Discussion

Method Development

When analyzing endogenous compounds in complex biological matrices, optimal compound separation is imperative. While the panel here consisted of 9 compounds, there are dozens of known endogenous compounds to monitor to ensure analysis was selective and accurate. In the initial method development, a 50 mm column was evaluated, however, isobars were nearly impossible to separate. For example, DHEA and testosterone are isobars (both with a molecular weight of 288.42 g/mol). To achieve baseline separation, a 100 mm column was necessary. A second set of isobars includes three isomers; 11-deoxycortisol, 17-deoxycortisol and 21-deoxycortisol. These three co-eluted and required isocratic separation. Without incorporation of an isocratic plateau (0.5 - 3.7 min) and using a longer column (100 mm), separation of the isobars proved to be unachievable. 17-Deoxycortisol and 21-dexoycortisol were not evaluated further in the analysis. Refer to **Figure 5** for chromatographic separation achieved by this method (LC method in **Table 1).**

Table 1. Instrument Parameters and LC Method

Instrument:	Agilent 1290 and AB SCIEX Triple Quad™ 6500+
Column:	Ascentis® Express C18, 10 cm x 3 mm I.D., 2.7 µm, with attached Ascentis® Express C18 guard cartridge, 5 mm x 3 mm I.D., 2.7 µm
Mobile Phase A:	10 mM ammonium formate + 0.05% formic acid in water, pH 3.
Mobile Phase B:	50:50 methanol:acetonitrile
Gradient/Flow rate:	

Time (min)	%A	%B	Flow Rate (µL/min)
0	50	50	500
0.5	45	55	400*
3.7	45	55	500
3.8	20	80	500
6.0	10	90	500
9.5	0	100	500
12.0	0	100	500
12.1	50	50	500
17.0	50	50	500
Column Temp:	40°C		
Detector:	MS/MS (see settings **Table 2 & 3**)		
Injection Volume:	15 µL		

* Slower flow rate allowed for the separation of 11-deoxycortisol from 17-deoxycortisol

Table 2. MS Parameters

Source/Gas Parameters	
Curtain Gas (CUR):	35
Collision Gas:	8
IonSpray Voltage (IS):	4500
Temperature (TEM):	600° C
Ion Source Gas 1 (GS1):	60
Ion Source Gas 2 (GS2):	60

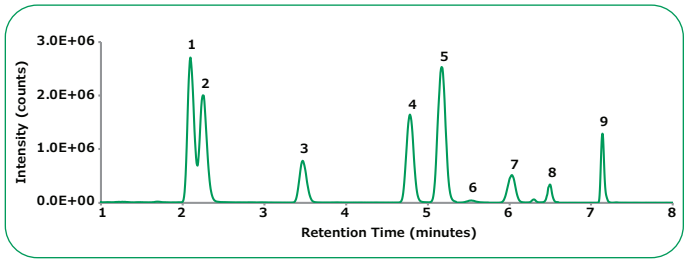


Figure 5. Representative TIC of a spiked blank serum with 9 steroids.
1 cortisone (50 ng/mL), **2** cortisol (50 ng/mL),
3 11-deoxycortisol (5 ng/mL), **4** testosterone (5 ng/mL),
5 androstenedione (5 ng/mL), **6** DHEA (5 ng mL),
7 17α-hydroxyprogesterone (5 ng/mL),
8 DHT (5 ng/mL), **9** progesterone (5 ng/mL).

Table 3. Transitions monitored by MS/MS

#	Analyte	RT (min)		Q1	Q3	Dwell Time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
1	Cortisone	2.14	Quant	361.2	163.1	80	140	10	33	18
			Qual	361.2	90.9	80	140	10	87	12
	Cortisone-$^{13}C_3$	2.14	IS	364.1	165.9	80	140	10	38	11
2	Cortisol	2.30	Quant	363.2	121.1	80	105	10	24	10
			Qual	363.2	91.1	80	105	10	72	10
	Cortisol-$^{13}C_3$	2.30	IS	366.1	124.2	80	105	10	31	10
3	11-Deoxycortisol	3.56	Quant	347.2	109.1	80	156	10	31	10
			Qual	347.2	97.1	80	156	10	29	8
	11-Deoxycortisol-D_5	3.53	IS	352.2	100.0	80	156	10	31	10
4	Androstenedione	5.25	Quant	287.1	97.1	120	80	10	30	6
			Qual	287.1	109.1	120	80	10	30	10
	Androstene-3,17-dione-2,3,4-$^{13}C_3$	5.25	IS	290.2	100.1	120	171	10	27	14
5	Testosterone	4.87	Quant	289.2	97.3	120	100	10	31	10
			Qual	289.2	108.8	120	100	10	31	12
	Testosterone-2,3,4-$^{13}C_3$	4.86	IS	292.2	99.9	120	196	10	29	12
6	Dehydroepiandrosterone (DHEA)	5.53	Quant	289.1	253.3	400	60	10	18	12
			Qual	289.1	213.2	400	60	10	18	12
	Dehydroepiandrosterone-D_5 (DHEA-D_5)	5.53	IS	294.2	276.3	400	151	10	13	8
7	17α-Hydroxyprogesterone (OH-progesterone)	6.10	Quant	331.4	109.2	200	100	10	40	10
			Qual	331.4	97.1	200	100	10	40	8
	OH-progesterone-2,3,4-$^{13}C_3$	6.09	IS	334.2	100.1	200	231	10	29	12
8	5α-Dihydrotestosterone (DHT)	6.55	Quant	291.2	255.2	180	110	10	24	10
			Qual	291.2	77.1	180	158	10	80	10
	5α-Dihydrotestosterone-D_3 (16,16,17-D_3)	6.52	IS	294.2	258.2	180	156	10	23	14
9	Progesterone	7.18	Quant	315.1	96.9	120	160	10	15	10
			Qual	315.1	109.0	120	160	10	15	14
	Progesterone-D_9	7.14	IS	324.2	99.9	120	201	10	29	10

Method Repeatabilty

A three-day precision and accuracy study was performed for 8 analytes utilizing external quality control serum from UTAK and NIST-971a (UTAK Laboratories, Inc., Valencia, CA, USA and NIST, Gaithersburg, MD, USA). Neither source offered verified values for DHT, therefore it was omitted. Ultimately, the inter-day precision of the 8 analytes (excluding DHT) varied from 0.30% to 12%. Intra-day precision ranged from 1.9% to 8.5%. Samples were performed in triplicates over three days.

Table 4. Real patient sample validation with UTAK Quality Controls. Concentration Range correspond to values from standards. Average Concentrations correspond to the experimental values obtained with Supel™ Swift HLB DPX Tips. %CVs are representative of 9 total replicates.

UTAK Quality Control Samples		Low (ng/mL)	High (ng/mL)
Progesterone	Concentration Range	7.5-12	20-24
	Average Concentration (%CV)	6.55 (6.5%)	17.9 (6.4%)
OH-Progesterone	Concentration Range	3-4.49	8-9.2
	Average Concentration (%CV)	3.9 (3.3%)	9.37 (5.5%)
Testosterone	Concentration Range	3-3.99	8-8.37
	Average Concentration (%CV)	3.49 (5.2%)	9.23 (7.3%)
Androstenedione	Concentration Range	1.5-1.74	4-4.38
	Average Concentration (%CV)	1.42 (0.4%)	4.03 (4.7%)
Cortisone	Concentration Range	15	40
	Average Concentration (%CV)	17.6 (3.4%)	49.4 (6.8%)
Cortisol	Concentration Range	60-65	180-200
	Average Concentration (%CV)	66.3 (3.9%)	226 (6.4%)
11-Deoxycortisol	Concentration Range	1-1.06	3-3.61
	Average Concentration (%CV)	1.05 (6.1%)	3.17 (2.3%)
DHEA	Concentration Range	3-3.8	8-11
	Average Concentration (%CV)	3.55 (9.5%)	9.67 (0.39%)

Using the data summarized in **Table 4, Figures 6** and **7** were created to compare theoretical UTAK versus measured values. A correlation graph comparing the UTAK provided values to the InTip™ dispersive SPE is shown in **Figure 6**. Overall, a slightly higher average steroid concentration was found using Supel™ Swift HLB DPX compared to the UTAK provided values with a slope of 1.19 with excellent linearity represented by R^2 = 0.9974 when considering all controls sampled. Another representation of this data is presented in **Figure 7** using the Bland-Altman analysis. The near zero bias (-3.02) and the evenly scattered error (positively and negatively) demonstrates that the two methods are interchangeable.

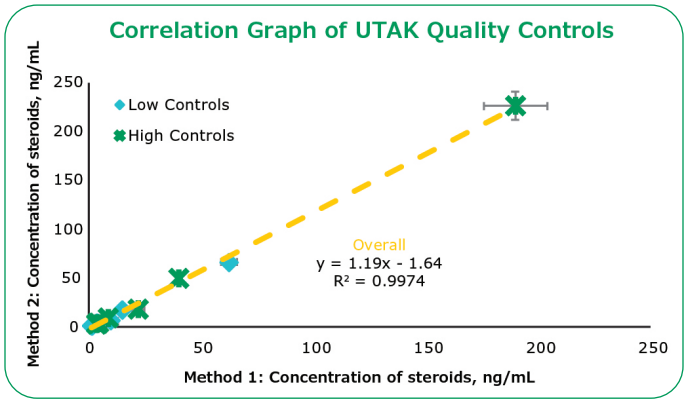


Figure 6. Correlation Graph of the Total Steroid Concentration comparing the two different approaches for the eight hormones (progesterone, OH-progesterone, testosterone, androstenedione, cortisone, cortisol, 11-deoxycortisol, DHEA). Method 1 corresponds to the UTAK provided values and Method 2 corresponds to using Supel™ Swift HLB DPX Tips. Low Controls: y = 1.07x - 0.44, R^2 = 0.9953, High Controls: y = 1.20x - 1.70, R^2 = 0.9985.

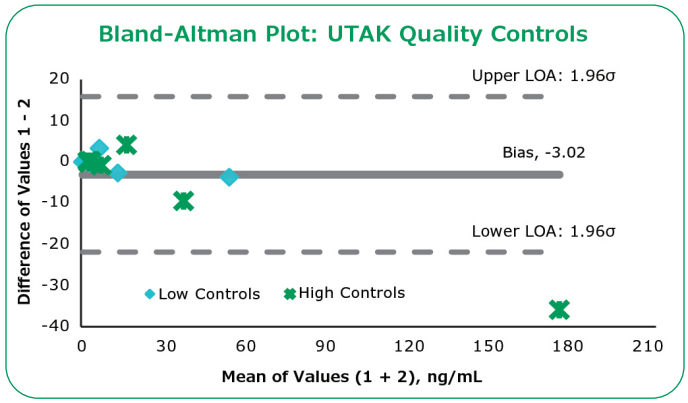


Figure 7. Total Steroid Concentration across eight different hormones simultaneously determined. Method 1 corresponds to the UTAK provided values and Method 2 corresponds to using Supel™ Swift HLB DPX Tips. The dash lines represent a 95% confidence interval.

Table 5. Real patient sample validation with NIST Quality Controls. Percent coefficients of variation (%CV) are representative of 9 total replicates.

NIST Quality Control Samples		Female (ng/mL)	Male (ng/mL)
Progesterone[a]	Concentration	2.63	0.0421
	Average Concentration (%CV)	2.41 (8.3%)	0.0416 (6.8%)
OH-Progesterone[b]	Concentration	0.8725	0.9635
	Average Concentration (%CV)	0.981 (3.4%)	1.09 (2.8%)
Testosterone[a]	Concentration	0.3231	5.808
	Average Concentration (%CV)	0.366 (5.3%)	6.52 (4.9%)
Androstenedione[b]	Concentration	0.8103	0.5359
	Average Concentration (%CV)	0.759 (0.2%)	0.498 (3%)

[a]The testosterone and progesterone levels were certified values via isotopic dilution(ID)-LC-MS/MS (NIST SRM971a).

[b] The "non-certified" values, OH-progesterone and androstenedione, were certified by Center of Desease Control (CDC) via ID-LC-MS/MS as well, however they were not certified by multiple sources outside the CDC.

Like with the UTAK data, using the data summarized in **Table 5**, **Figures 8** and **9** were created. A correlation graph comparing the NIST values to the InTip™ dispersive solid phase extraction (dSPE) is shown in **Figure 8**. Overall, a slightly higher average steroid concentration was shown using Supel™ Swift HLB DPX Tips compared to the NIST provided values with a slope of 1.11 with excellent linearity represented by R^2 = 0.9922 with a near zero y-intercept. Another representation of this data is presented in **Figure 9** using the Bland-Altman analysis. The near zero bias (-0.09) and the evenly scattered error (positively and negatively) demonstrates once again that the two methods are interchangeable.

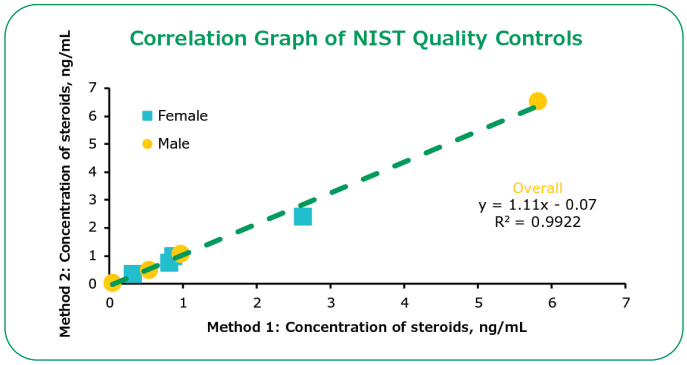


Figure 8. Correlation graph of the Total Steroids Concentration comparing the two different values for the four hormones (progesterone, OH-progesterone, testosterone, and androstenedione). Method 1 corresponds to the NIST values and Method 2 corresponds to using Supel™ Swift HLB DPX Tips. Female Controls: y = 0.88x + 0.11, R^2 = 0.9934, Male Controls: y = 1.13x - 0.04, R^2 = 1.00.

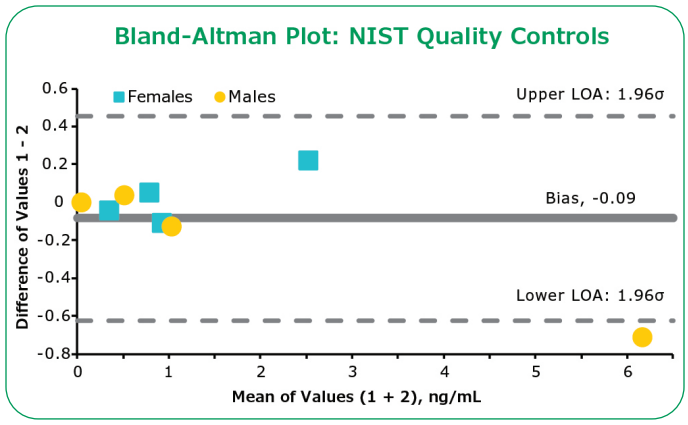


Figure 9. Total Steroid Concentration across four different hormones simultaneously determined. Method 1 corresponds to the NIST values and Method 2 corresponds to using Supel™ Swift HLB DPX Tips. The dash lines represent a 95% confidence interval.

Great sensitivity and chromatographic separations allowed for levels of detection in the sub-nanogram per milliliter range. Limits of quantification (LOQ) range below the lowest calibrator level at 0.025 ng/mL for all analytes except cortisone and cortisol, which had a lowest calibrator of 0.25 ng/mL. The LOQ was calculated based on a signal-to-noise above 10 and the limit of detection (LOD) was based on a signal-to-noise above 3. In all cases, the reproducibility at the lowest calibrator is well within the Bioanalytical Validation Guidelines (BAVG)[6] of 15% which corresponds with the non-lowest accepted calibrator **(Table 6)**. According to the BAVG, the accepted reproducibility criteria for the lowest calibrator is 20%.

Table 6. The signal-to-noise (S/N) and reproducibility for each analyte at the lowest calibrator tested. Percent coefficients of variation (%CV) are represented from triplicate calibration curves over three days.

Analyte	Level 1 Concentration (ng/mL)	S/N	% CV
Cortisone	0.25	229	10%
Cortisol	0.25	98.4	4%
11-Deoxycortisol	0.025	18.9	6%
Testosterone	0.025	80.8	7%
Androstenedione	0.025	34.8	3%
DHEA	0.025	23.6	8%
OH-Progesterone	0.025	31.2	14%
DHT	0.025	11.6	8%
Progesterone	0.025	19.4	5%

Using the SWGTOX guidelines and a total of eight replicates for each analyte, the recoveries and the matrix effects for each analyte were determined **(Table 7).** Using **Eq 1**, the recovery of the method was determined in the range of 65-86% with an average of 71% recovery. The influence of matrix was determined using **Eq 2** with an average of 33% ionization suppression.

Eq 1.

$$Recovery = \frac{Spiked\ sample\ prior\ to\ extraction}{Spiked\ sample\ post\text{-}extraction} \times 100\%$$

Eq 2.

$$Ionization\ Effects = \frac{Spiked\ sample\ post\text{-}extraction}{Neat\ sample\ in\ solution} \times 100\%$$

Table 7. Recovery and matrix effects. Values found using SWGTOX guidelines.

Analyte	Average Recovery (n=9)	% RSD	Matrix Effects (n=8)*
Cortisone	72%	10%	29%
Cortisol	70%	10%	30%
11-Deoxycortisol	69%	9%	40%
Androstenedione	67%	9%	34%
Testosterone	69%	10%	30%
DHEA	86%	10%	12%
OH-Progesterone	67%	9%	41%
DHT	70%	12%	38%
Progesterone	65%	8%	46%

*Matrix effects are defined as: (+) positive indicates ion suppression, and a (-) negative indicates ion enhancement.

Conclusion

The use of Supel™ Swift HLB DPX Tips for the analysis of various steroids in blood serum was shown to be reproducible across two different standards (UTAK and NIST) and offers an alternative that is faster and programmable for clinical testing laboratories. This method provides the necessary sensitivity relevant to clinical values while also enabling the ability for high throughput sample processing for fast turnaround times. The accurate and sensitive method described here can be a valuable tool for quantification of steroids in serum.

References:

1. Supel™ Swift DPX HLB Tips Datasheet T721005
2. Nieman, Lynnette K et al. "The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline." The Journal of clinical endocrinology and metabolism vol. 93,5 (2008): 1526-40. doi:10.1210/jc.2008-0125
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6. Food and Drug Administration. (2018) Bioanalytical Method Validation Guidance for Industry. Washington, DC: Center for Drug Evaluation and Research

Featured Products

Description	Cat. No.
Supel™ Swift HLB DPX Tips 3 mg microelution Hamilton® 300 µl	**53001-U**
Reference Materials & Solutions	
DDC Mass Spect Gold® Serum	**MSG3000**
Androstenedione solution - 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**A-075**
Androstene-3,17-dione-2,3,4-$^{13}C_3$ solution - 100 µg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**A-084**
Cortisone solution - 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-130**
Cortisone-$^{13}C_3$ solution - 100 µg/mL in methanol, certified reference material, ampule of 1 mL, Cerilliant®	**C-160**
Cortisol solution - 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**C-106**
Cortisol-$^{13}C_3$ solution - 100 µg/mL in methanol, certified reference material, ampule of 1 mL, Cerilliant®	**C-216**
11-Deoxycortisol solution - 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**D-061**
11-Deoxycortisol-D_5 (2,2,4,6,6-D_5) solution - 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**D-078**
Dehydroepiandrosterone (DHEA) solution - 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**D-063**
Dehydroepiandrosterone-D_5 (DHEA-D_5) (2,2,3,4,4-D_5) solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**D-064**
5α-Dihydrotestosterone (DHT) solution - 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**D-073**
5α-Dihydrotestosterone-D_3 (16,16,17-D_3) solution - 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**D-077**
17α-Hydroxyprogesterone solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**H-085**
17α-Hydroxyprogesterone-2,3,4-$^{13}C_3$ solution 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**H-100**
Progesterone solution - 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**P-069**
Progesterone-D_9 solution - 100 µg/mL in methanol, ampule of 1 mL, certified reference material, Cerilliant®	**P-070**
Testosterone solution - 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**T-037**
Testosterone-2,3,4-$^{13}C_3$ solution - 100 µg/mL in acetonitrile, ampule of 1 mL, certified reference material, Cerilliant®	**T-070**
HPLC, Solvents & Reagents	
Ascentis® Express C18, 2.7 µm HPLC Column L × I.D. 10 cm × 3 mm	**53814-U**
Ascentis® Express Guard Cartridge Holder	**53500-U**
Ascentis® Express C18, Guard Cartridge 2.7 µm particle size, L × I.D. 5 mm × 3 mm, pkg of 3 ea	**53504-U**
Formic acid 98-100%, LC-MS LiChropur™	**5.330002**
Methanol, LC-MS LiChrosolv®	**1.03726**
Acetonitrile, LC-MS LiChrosolv®	**1.00029**
Water, LC-MS LiChrosolv®	**1.15333**
Suggested Consumables	
Zone-Free™ Sealing Films	**Z721646**
SealPlate Film	**Z369659**
Nunc™ 96 DeepWell™ plate, non-treated size 96 wells, U-bottom natural polypropylene wells, maximum volume 2 mL, non-sterile	**Z717266**
Corning® 96 well Polypropylene Deep Well Plate size 96 wells, V-bottom clear, polypropylene, maximum volume 2 mL, non-sterile	**CLS3960**
BRAND® 96-well deep well plate Stackable volume 2.2 mL, polypropylene, non-sterile	**BR701354**
Axygen® Deep well plate size 96 wells, size 2.0 mL, non-sterile	**AXYPDW20C**

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