

High speed UHPLC-MS/MS determination of multiple steroids in human serum using the Nexera MX system for multiplex analysis

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Introduction

Steroid hormones play a crucial role in controlling metabolism, inflammation and immune functions. Changes in steroid profiling reflect disease status and help research into a number of disorders, including congenital adrenal hyperplasia (CAH), Cushing's disease and polycystic ovarian disease. To overcome many limitations of immunoassays, high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) has the potential to find its place in the clinical laboratory medicine for quantification of steroid hormones to profile steroids in a disease process. To enhance steroid profiling a highly sensitive analysis method for simultaneous determination of multiple steroids by UHPLC-MS/MS was developed to support clinical research.

To deliver a higher sample throughput the method used an autosampler capable of overlapping sample injections. The Shimadzu Nexera MX multiplex LCMS system has a Dual Stream Technology (MX-DST) using two separate analysis systems to perform overlapping sample injections. This design supports either an injection cycle of two batches of samples at the same time or to inject one batch of samples using the two separate streams of the Nexera MX system. This study also considers the influence on the results when using the two separate streams for one batch compared to a conventional approach of one analysis stream for one batch.

Materials and Methods

Sample Pretreatment

A synthetic surrogate serum (SigMatrix) was spiked with the steroids panel to prepare calibration and quality control samples. After addition of the respective internal standards the samples (550 μ L) were diluted 1:1 with ultrapure water and extracted using an Isoolute SLE+ cartridge (Biotage). The steroid panel was eluted from the Isoolute SLE+ cartridge using 5mL dichloromethane. The

dichloromethane was evaporated to dryness and reconstituted in 50 μ L of water/methanol (1:1 v/v) and transferred to a vial with glass insert stored at 5 °C prior to analysis. For this study calibration and QC samples containing final concentrations equal to the extracted samples were prepared.

Analytical conditions

Steroids were detected by LC-MS/MS using a Nexera MX and LCMS-8050/LCMS-8060 systems (Shimadzu Corporation, Japan). The Nexera MX Dual Stream Technology uses two independent analysis systems

(streams) to perform overlapping sample injections delivering increased sample throughput with a single MS/MS detector (Figure 1). Analytical conditions are reported in Table 1.

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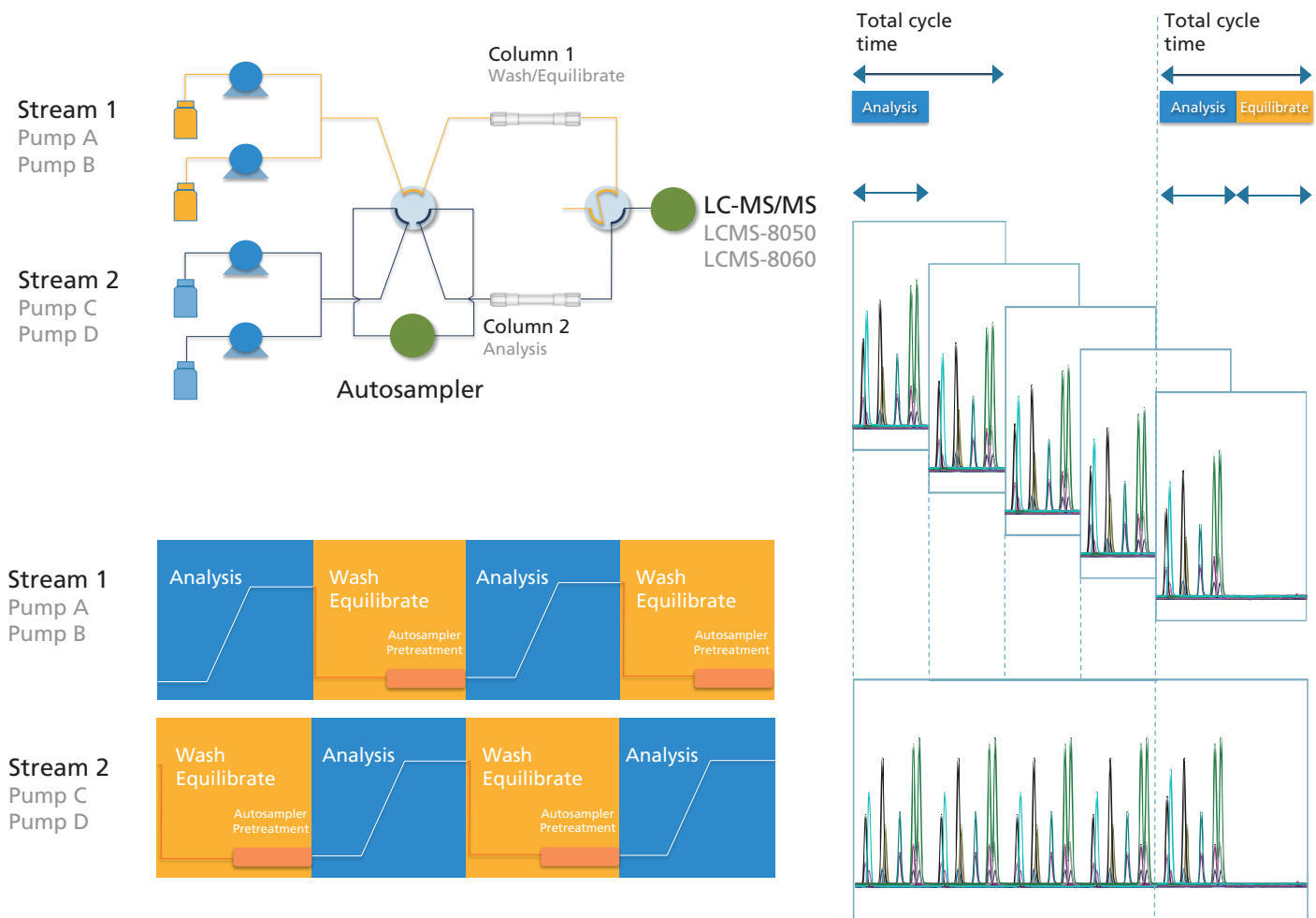


Figure 1 : Schematic view of overlap injection function in Nexera MX system

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Table 1 : LC-MS/MS acquisition method designed to accelerate sample throughput for steroid analysis.

Mass Spectrometry (MS/MS)			
MS system	: LCMS-8050/8060 (Shimadzu, Japan)		
Ionization	: HESI (positive/negative)		
Nebulizing Gas Flow	: 3.00 L/min (N ₂)		
Drying Gas Flow	: 10.0 L/min (N ₂)		
Heating Gas Flow	: 10.0 L/min (Air)		
Desolvation line	: 150 °C		
Block Temperature	: 500 °C		
Interface Temperature	: 400 °C		
Dual Stream LC			
LC System	: Nexera MX (Shimadzu, Japan)		
Analytical Column	: Restek Raptor Biphenyl 2.7 μm, 50 x 3 mm (2x)		
Mobile Phase A/C	: Water + additive		
Mobile Phase B/D	: Methanol + additive		
Injection volume	: 30 μL		
Column Temperature	: 30 °C		
Dual Stream Gradient (the streams are automatically changed in a running sequence to maximize throughput)			
Stream 1	:		
	Time (min)	Flow (mL/min)	B.Conc %
	0.00	0.800	70
	1.00	0.800	73
	1.50	0.800	73
	3.80	0.800	97.8
Stream 2	:		
	Time (min)	Flow (mL/min)	D.Conc %
	0.00	0.800	97.80
	0.20	0.800	100
	0.70	0.800	100
	0.80	0.800	70
	0.81	0.100	70
	2.24	0.100	70
	2.25	0.800	70
Autosampler cut-off	: 2.25min		

Results

The original method for simultaneous determination of multiple steroids (Table 2) by UHPLC-MS/MS was successfully used with the Shimadzu Nexera MX multiplex LCMS system. Results for calibration curves and QC samples obtained from a batch using both streams where comparable to those obtained from a single stream.

Exemplary calibration curves are shown in Figure 2. QC results for 17β-Estradiol and Androstenedione are shown in Table 3. A chromatogram overlay of multiple injections using the two streams for exemplary chromatograms are shown in Figure 3.

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Table 2 : LOQ and calibration ranges

Steroid	Internal Standard	LOQ (pg/mL)	Linear Range
Aldosterone	D7-Aldosterone	2	0.002-15 ng/mL
Estradiol	D5-Estradiol	1	0.001-15 ng/mL
Testosterone	¹³ C3-Testosterone	2	0.002-15 ng/mL
5a-Dihydrotestosterone	D3-5a-Dihydrotestosterone	25	0.025-15 ng/mL
11-Deoxycortisol	D5-11-Deoxycortisol	1	0.001-15 ng/mL
11-Deoxycorticosterone	D5-11-Deoxycortisol	1	0.001-7.5 ng/mL
Corticosterone	D5-11-Deoxycortisol	1	0.001-7.5 ng/mL
Cortisol	D4-Cortisol	1	0.001-15 ng/mL
Cortisone	D4-Cortisol	5	0.005-15 ng/mL
17-Hydroxyprogesterone	¹³ C3-17-Hydroxyprogesterone	1	0.001-7.5 ng/mL
Androstenedione	¹³ C3-Androstenedione	1	0.001-15 ng/mL
Progesterone	D9-Progesterone	0.5	0.0005-7.5 ng/mL
Estrone	D5-Estrone	0.5	0.0005-15 ng/mL
DHEA	D5-DHEA	10	0.01-15 ng/mL
DHEA sulfate	D5-DHEA sulfate	100	0.01-1500 ng/mL

Calibration Curves

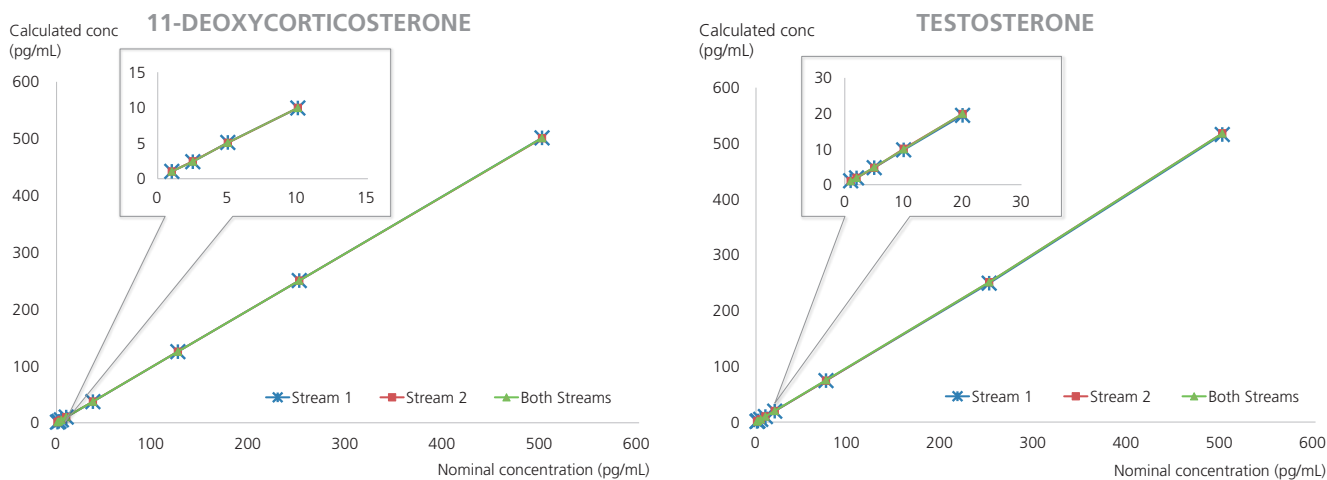


Figure 2 : Overlay of three calibration curves for 11-deoxycorticosterone and testosterone obtained from single stream 1 and 2 and from both streams.

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Table 3 : Analysis results for QC samples

17β-Estradiol					Androstenedione				
		Stream 1	Stream 2	Both Streams			Stream 1	Stream 2	Both Streams
Quality Control 50pg/mL	Conc (pg/mL)	47.71	47.65	51.5	Quality Control 50pg/mL	Conc (pg/mL)	55.6	53.6	54.4
	Accuracy (%)	95.43	95.3	103		Accuracy (%)	111.2	107.3	108.8
	RSD (%)	5.22	4.92	3.3		RSD (%)	5.37	1.00	3.83
Quality Control 100 pg/mL	Conc (pg/mL)	97.97	96.4	101	Quality Control 100 pg/mL	Conc (pg/mL)	103.8	103.1	102.9
	Accuracy (%)	97.97	96.58	101.4		Accuracy (%)	107.6	102.6	106.3
	RSD (%)	4.2	2.48	0.92		RSD (%)	3.29	1.53	4.54

Representative chromatograms

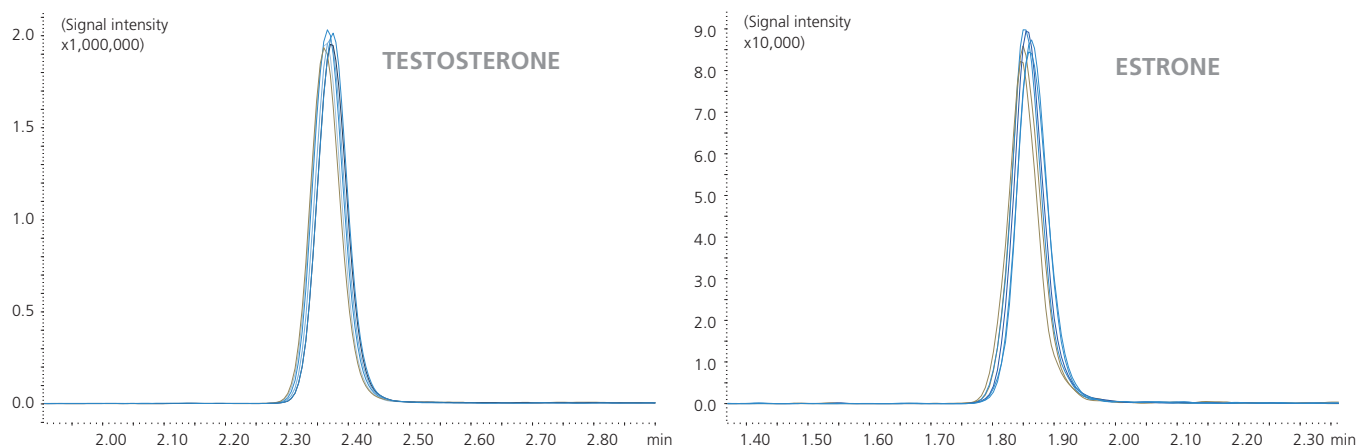


Figure 3 : Overlay of 6 mass chromatograms of Testosterone and Estrone (100pg/mL) using automatic switching between both streams to maximize productivity without compromising data

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Conclusion

Transferring a highly optimized LC-MS/MS method for steroids from a conventional single stream system to a dual stream multiplex LC/LC-MS/MS platform significantly accelerated sample cycle times without affecting data quality. This approach has several advantages as it

increases sample throughput using the same LC platform and footprint, maximizes data acquisition times on the MS and is simple to use as the software is designed for dual stream analysis.



Nexera MX with LCMS-8060 triple quadrupole mass spectrometer

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