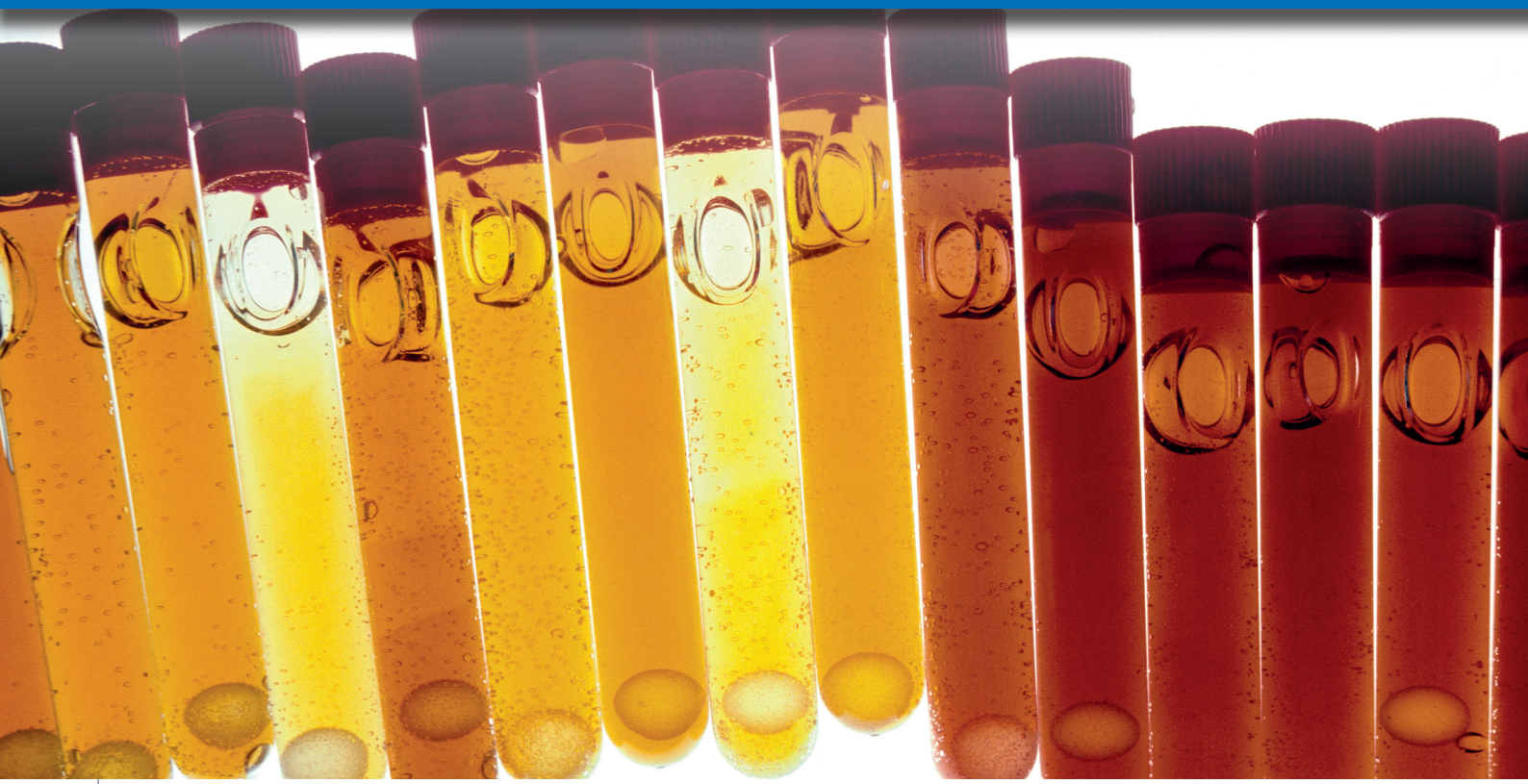

Quantitation of Steroids in Serum by UHPLC-Triple Quadrupole Mass Spectrometry

This study demonstrates a simple, rapid, and reliable method for the simultaneous quantitation of 8 steroid hormones and their metabolites in serum using the Bruker Elute™ UHPLC coupled to the EVOQ™ LC-TQ Elite MS/MS system. Sample preparation was performed via protein precipitation using a ClinMass® Complete Kit for Steroids in Serum/Plasma.

Introduction

Steroids are biologically active compounds found in animals, plants, and fungi. They can act as hormones, serving as neuro-endocrine regulators of both physiological development and critical bodily functions, including metabolic control, stress and immune response, and maintenance of water and mineral balance. All steroids are fat-soluble lipids which pass through cell membranes to be distributed through the blood stream, and they share a core structure of three cyclohexane rings and a cyclopentane ring.

Keywords: Steroids, serum, quantitation

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Steroid hormones are divided into two broad classes, corticosteroids and sex steroids, with sub-classifications based on the receptors to which they bind. Corticosteroids include glucocorticoids (e.g., cortisol) and mineralocorticoids (e.g., aldosterone). Sex steroids include androgens (e.g., testosterone), estrogens (e.g., estradiol), and progestogens (e.g., progesterone). These classifications can refer to endogenous steroid hormones naturally produced from cholesterol or synthetic hormones produced as pharmaceutical medications.

Many genetic disorders are related with abnormalities in steroid hormone production, and other disease states (or treatments for the disease) often have indirect effects on the endocrine system. Dramatic shifts in steroid hormone levels can also be observed during growth, maturation, and aging processes, or from environmental exposure to endocrine disrupting chemicals (EDCs) or steroid abuse. The accurate quantitation of steroid hormone levels, particularly as affected by a disease state or the administration of therapeutics, is a key element of modern clinical research.

The method described in this manuscript focuses on the rapid and reliable quantitation of steroid hormones in human serum by UHPLC-triple quadrupole mass spectrometry.

Experimental

The analysis was performed on an Bruker EVOQ™ LC-TQ Elite system coupled to an Elute UHPLC using the ClinMass® Complete Kit for Steroids in Serum/Plasma (RECIPE Chemicals + Instruments GmbH, Munich, Germany, MS12000) which included the mobile phase, autosampler washing solution, precipitation reagent, and the HPLC column.

Table 1: Mass Spectrometry Method Conditions

Liquid Chromatography			
Instrument	Bruker Elute UHPLC		
Column	ClinMass MS12030 (RECIPE)		
Mobile Phase A	Included within the ClinMass MS12000 Kit (RECIPE)		
Mobile Phase B	Included within the ClinMass MS12000 Kit (RECIPE)		
Gradient	Time [min]	Mobile Phase B [%]	Flow rate [ml/min]
	0.00	27	0.5
	4.50	57	0.5
	4.51	100	0.5
	4.64	100	0.5
	4.75	100	0.8
	5.35	100	0.8
	5.36	27	0.8
	5.65	27	0.8
	5.66	27	0.5
	6.00	27	0.5
Injection Volume	20 µL		
Column Oven	40°C		
Mass Spectrometry			
Instrument	Bruker EVOQ™ LC-TQ Elite		
Ion Source	VIP H-ESI positive and negative, 4500 V		
Probe Gas	50 units at 450°C		
Cone Gas	20 units at 350°C		
Nebulizing Gas	50 units		
Active Exhaust	on		
Collision Gas	Argon, 1.5 mTorr		
MRM Transitions	see Table 2		

Table 2: Retention times, MRM transitions, calibration ranges, and RSD RF of calibration curves

Analyte	Retention time (min)	Precursor Ion	Product Ion 1	CE 1 (V)	Product Ion 2	CE 2 (V)	Calibration Range [µg/L]	RSD RF [%]
11-Deoxycortisol	3.18	347.1	109.2	25	97.2	23	0.100 – 13.4	6.8
21-Deoxycortisol	2.84	347.2	311.2	14	121.1	24	0.117 – 14.7	11.7
17-Hydroxyprogesterone	4.56	331.2	97.2	21	109.1	24	0.109 – 12.5	4.2
21-Hydroxyprogesterone	4.38	331.2	97.2	20	109.2	25	0.196 – 24.4	9.0
Androstendione	4.37	287.1	97.2	20	109.2	22	0.117 – 12.7	3.8
Cortisol	2.08	363.2	121.1	30	97.1	33	2.75 – 325	4.4
DHEAS*	2.64	367.1	97.1	27	80.2	73	43.9 – 4851	2.2
Testosterone	4.19	289.2	97.2	22	109.2	24	0.099 – 11.0	4.9

**DHEAS measured in ESI negative mode, all other analytes in ESI positive mode*

Samples and Sample Preparation

Serum calibrators (MS12013) and three ClinChek® quality control samples (low, medium, high concentration; MS12083) were provided by RECIPE. In addition, the serum sample of a test individual (healthy female, 33 years old) was analyzed. The quality control samples and the serum test sample were measured in quadruplicate as technical replicates.

Following the protocol of the ClinMass® kit, 50 µL serum samples were added to 75 µL precipitation reagent containing the isotopically labelled internal standards and vortexed for 1 minute. After precipitation for 10 minutes and a 10 second vortex, the samples were centrifuged for 10 minutes at 14,000 x g. The supernatants then were transferred to HPLC vials for analysis by UHPLC-MS/MS.

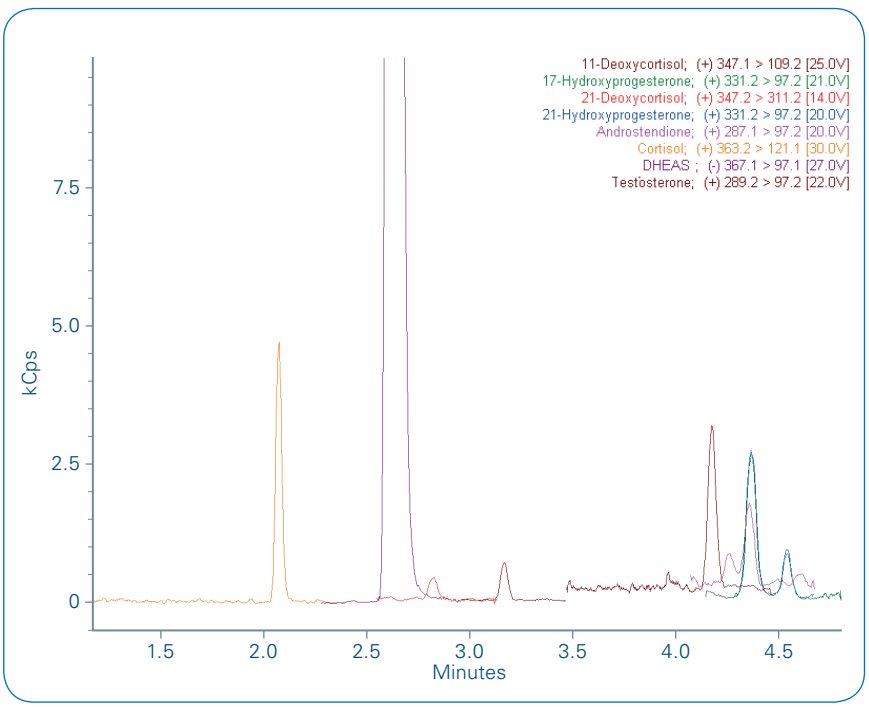

Figure 1: Overlaid MRM traces of all analytes (lowest calibrator level)

Results and Discussion

Following fast and simple sample preparation requiring only 50 µL of serum, the chromatographic separation of the eight analytes was performed within 6.0 minutes using the Elute UHPLC system. Figure 1 illustrates an overlay of the MRM traces for all measured analytes.

The quantitation of the analytes was performed using six isotopically labelled internal standards. Calibration curves included six calibrator levels and provided excellent linearity with RSD RF values from 2.2 – 11.7 % (Table 2). In a perfect calibration curve, the RF (response factor = relative area/amount) is the same for each level. The RSD [%] of the RF values of all calibrators is useful to

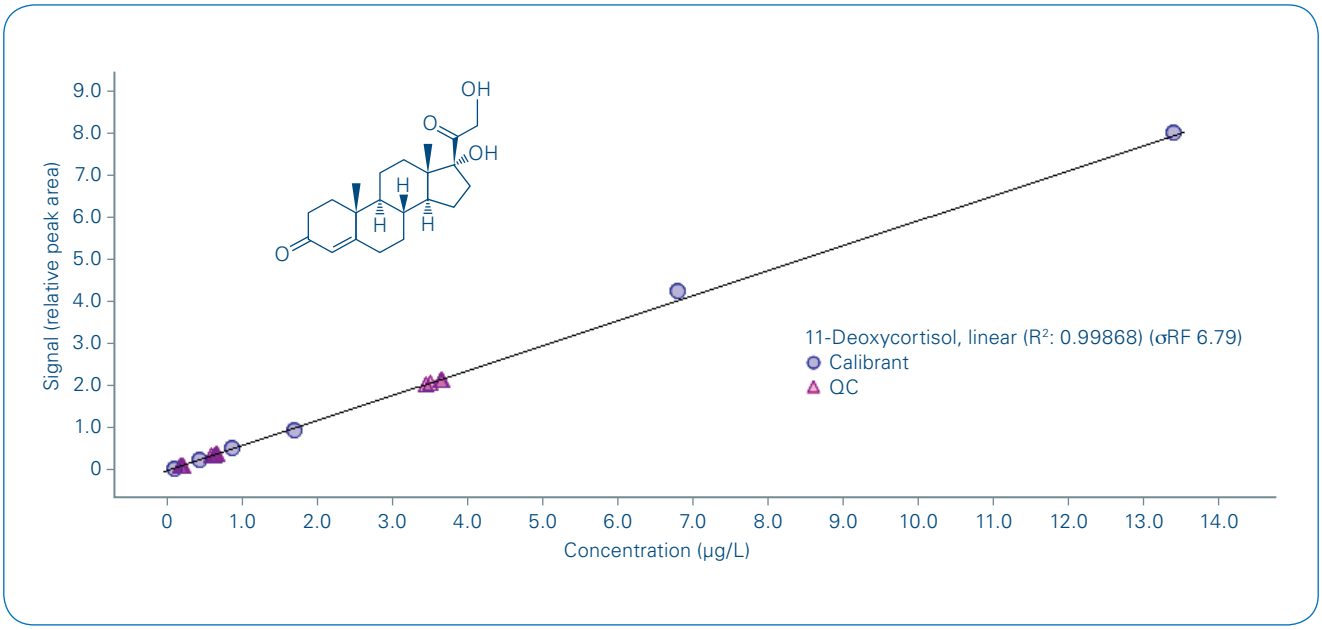

Figure 2: Calibration curve of 11-Deoxycortisol. Calibration range 0.1 – 13.4 µg/L

evaluate the quality of a calibration curve. Acceptable values for RSD RF are < 15 – 20%. The calibration curve of 11-Deoxycortisol is shown as an example in Figure 2.

Quality controls (QC) in serum with low (QC I), middle (QC II) and high (QC III) concentrations, as well as a serum sample of a test individual were measured four times. The RSD of ≤ 9% for all analytes underlines the very good precision of the instrumentation used. The experiments also showed a high accuracy with a bias within ±9% for 91% of all determinations (max. value 13.2%). Detailed results are presented in Table 3. The values for precision and accuracy are well within the range required by common guidelines for quantitative results. In the sample of the test individual, all targeted analytes could be detected, and all quantitative results were within reference ranges reported in the literature.

Table 3: Quantitative results, bias and relative standard deviation of fourfold measurement of Quality Controls I – III and test sample

Sample	QC I			QC II			QC III			Test Sample	
Analyte	**Actual Value [µg/L]**	**Bias [%]**	**RSD [%]**	**Actual Value [µg/L]**	**Bias [%]**	**RSD [%]**	**Actual Value [µg/L]**	**Bias [%]**	**RSD [%]**	**Actual Value [µg/L]**	**RSD [%]**
11-Deoxycortisol	0.204	-2.3	5.9	0.644	-0.4	4.8	3.58	0.1	2.9	0.304	2.1
21-Deoxycortisol	0.237	3.2	5.7	0.752	9.0	5.8	4.18	13.2	3.6	< LOQ	-
17-Hydroxyprogesterone	0.197	-6.2	5.0	0.571	-6.4	3.5	3.46	2.3	3.8	1.57	2.6
21-Hydroxyprogesterone	0.323	-11.3	2.5	1.13	7.2	5.1	5.98	-0.5	9.0	< LOQ	-
Androstendione	0.223	-4.3	8.1	0.613	-0.2	2.3	3.19	-1.3	5.4	1.27	8.8
Cortisol	5.18	6.2	2.2	14.5	-0.1	1.1	79.9	-0.2	2.4	124	1.9
DHEAS	79.0	-4.4	2.4	224	-0.9	2.4	1240	1.1	0.5	1853	2
Testosterone	0.193	-0.1	2.6	0.534	-1.6	3.5	3.03	4.4	2.1	0.395	2.2

Conclusions

- The Bruker Elute UHPLC coupled to the Bruker EVOQ™ LC-TQ Elite system and the ClinMass® Complete Kit for Steroids in Serum/Plasma provide a quick and reliable method to easily detect and quantitate eight steroids in serum.
- Low sample requirements (50 µL serum), easy preparation, and short run time (6.0 minutes) support high sample throughput. Linearity of calibration, precision, and accuracy were outstanding, supporting the use of this combined system in clinical research workflows.

Further Reading

To learn more about the Bruker EVOQ™ LC-TQ Elite and Elute LC systems, please see:
https://www.bruker.com/products/mass-spectrometry-and-separations/lc-ms/evoq/overview.html
https://www.bruker.com/products/mass-spectrometry-and-separations/lc-ms/liquid-chromatography/elute-lc-series/overview.html

Please see Application Notes LCMS-138, LCMS-139, LCMS-145, LCMS-146, and LCMS-147 for examples of quantitation of clinically relevant drug panels using the ClinMass TDM Platform.

Acknowledgement

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