

LC-MS/MS Method for the Determination of Steroids from Urine Using SOLA and Core Enhanced Technology Accucore HPLC Column.

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Key Words

- SOLA Cartridges and Plates
- Accucore
- Solid Core
- Core Enhanced Technology
- Steroids
- Hydrocortisone
- Cortisone
- Corticosterone
- 11- α hydroxyprogesterone
- Betamethasone

Abstract

This application note demonstrates the use of the Thermo Scientific SOLA well plates for the extraction of steroids from urine and the subsequent fast separation using a Thermo Scientific Accucore RP-MS column.

Introduction

SOLA™ products are a revolutionary new Solid Phase Extraction (SPE) product range. This first in class SPE product range introduces next-generation, innovative technological advancements, giving unparalleled performance characteristics compared to conventional SPE, phospholipid and protein precipitation products.

This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA products have significant advantages for the analyst when processing compounds in complex matrices particularly in high throughput bioanalytical and clinical laboratories where reduced failure rate, higher analysis speed and lower sample/solvent requirements are critical.

The increased performance from SOLA products provides higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Accucore™ HPLC columns use Core Enhanced Technology to facilitate fast and high efficiency separations. The 2.6 μ m diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 μ m diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 μ m materials.

Hydrocortisone, cortisone, corticosterone and 11- α hydroxyprogesterone belong to the progesterone class of steroid hormones. Progesterones are characterized by the 21-carbon backbone and are primarily used to maintain pregnancy. The extraction of these steroids from urine, their separation on an Accucore RP-MS column and their quantification by LC-MS/MS is demonstrated in this application note.



Experimental Details

Consumables	Part Number
Fisher Scientific LC-MS grade water	W/0112/17
Fisher Scientific LC-MS grade acetonitrile	A/0638/17
Fisher Scientific LC-MS grade formic acid	A117-50
Hydrocortisone, cortisone, corticosterone, 11- α hydroxyprogesterone and betamethasone (IS)	

Sample Preparation - SOLA	Part Number
Matrix:	urine
Cartridge type:	SOLA 96 well plate 60309-001
Conditioning stage:	1 mL methanol, 1 mL water
Application stage:	1 mL spiked urine containing internal standard for corticosterone and 11- α hydroxyprogesterone. 1 mL urine for hydrocortisone and cortisone
Washing stage:	200 μ L of 20:80 (v/v) methanol / water
Elution stage:	2 x 200 μ L methanol
Additional stage:	Evaporate to dryness under a gentle stream of nitrogen and reconstitute in 200 μ L water

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela	
Column:	Accucore RP-MS 2.6 μ m, 100 x 2.1 mm	17626-102130
Mobile phase:	A: water + 0.1% formic acid B: acetonitrile + 0.1% formic acid	
Gradient:	Time (minutes)	%B
	0.0	25
	0.02	25
	4.00	75
	4.50	75
	4.51	25
	6.00	25
Flow rate:	0.6 mL/min	
Column temperature:	25 °C	
Injection details:	2.5 μ L	
Weak Injection wash solvent:	20:80 (v/v/v) acetonitrile / water	
Strong injection wash solvent:	45:45:10 (v/v/v) acetonitrile / acetone / isopropanol	

MS Conditions

Instrumentation: Thermo Scientific TSQ Vantage

Ionization conditions	HESI
Polarity	+ve
Spray voltage (V)	3000
Vaporizer temp (°C)	300
Sheath gas pressure (Arb)	60
Aux gas pressure (Arb)	30
Capillary temp (°C)	300
Collision pressure(mTorr)	1.5
Scan time (s)	0.02
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7

Table 1. Vantage™ conditions

Compound	Hydro-cortisone	Cortisone	Corti-costerone	11- α -hydroxypro-gesterone	Beta-methasone (IS)
Precursor (m/z)	363.23	361.30	347.29	331.29	393.28
Product (m/z)	121.03	163.12	329.27	295.24	373.26
Collision energy (eV)	21	20	10	11	5
S-lens (RF voltage)	87	102	82	84	63

Table 2. Transition details

Data Processing

Software: Thermo Scientific LC QUAN

Results

As shown in Figure 1, the four steroids were separated in less than 3 minutes using an Accucore RP-MS column.

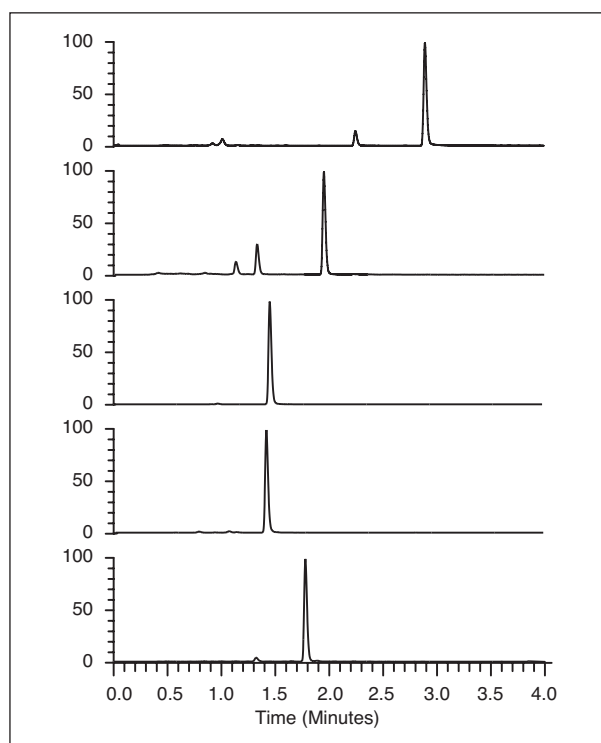


Figure 1. Extracted ion chromatograms of 11- α hydroxyprogesterone (1), corticosterone (2), cortisone (3), hydrocortisone (4) and internal standard betamethasone (5) from urine using SOLA well plate

Hydrocortisone results

Hydrocortisone standards prepared in water were linear over the dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9937 (Figure 2). As shown in Table 3, six replicates of urine were extracted using the SOLA 96 well plates and the endogenous level of hydrocortisone was found to be 17.3 ng/mL with precision of 11.1%.

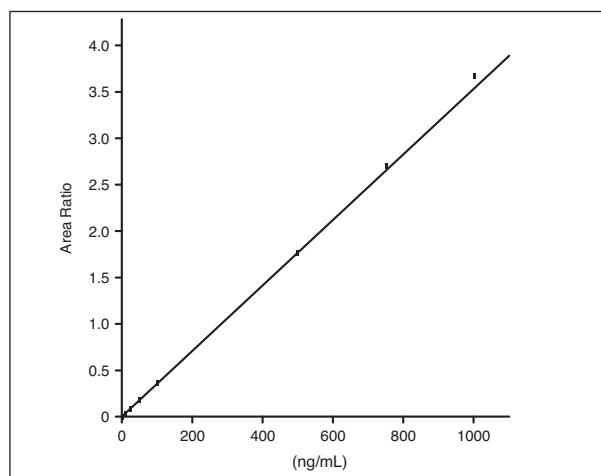


Figure 2. Linear dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9937 for hydrocortisone standards

Cortisone results

Cortisone standards prepared in water were linear over the dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9981 (Figure 3). As shown in Table 3, six replicates of urine were extracted using the SOLA well plates and the endogenous level of cortisone was found to be 55.6 ng/mL with precision of 7.4%.

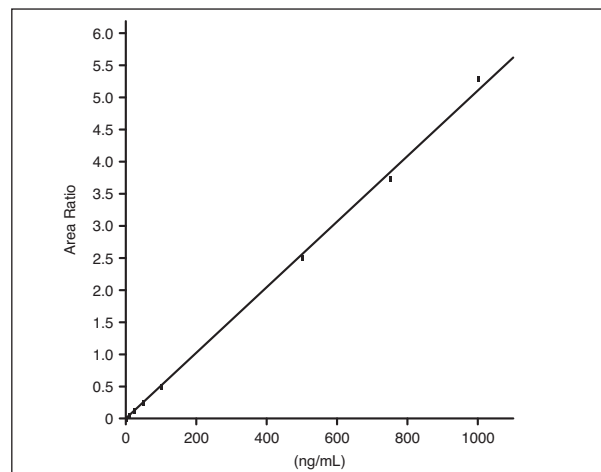


Figure 3. Linear dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9981 for cortisone standards

Compound	n	Average Endogenous Level (ng/mL)	Precision
Hydrocortisone	6	17.3	11.1 %
Cortisone	6	55.6	7.4 %

Table 3. Summary of hydrocortisone and cortisone results after extraction using SOLA well plates

Corticosterone results

Corticosterone standards prepared in water were linear over the dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9998 (Figure 4). QC samples were extracted in triplicate at both low and high concentrations of 15 ng/mL and 600 ng/mL. Precision for each QC level were less than three percent relative standard deviation (Table 4). Overspikes were run in duplicate at a concentration of 250 ng/mL and used to calculate the percentage recovery level for corticosterone of 131.2%. No carryover was observed for corticosterone (Table 5).

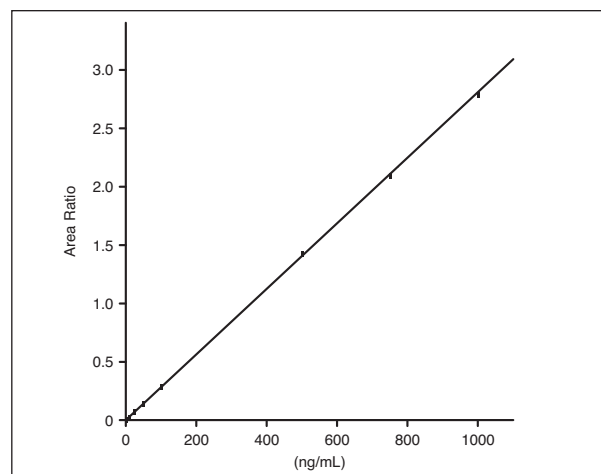


Figure 4. Linear dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9998 for corticosterone standards

11- α hydroxyprogesterone results

11- α hydroxyprogesterone standards prepared in water were linear over the dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9992 (Figure 5). QC samples were extracted in triplicate at both low and high concentrations of 15 ng/mL and 600 ng/mL. Precision for each QC level were less than three percent relative standard deviation (Table 4). Overspikes were run in duplicate at a concentration of 250 ng/mL and used to calculate the percentage recovery level for

11-alpha hydroxyprogesterone of 130.8%. No carryover was observed for 11- α hydroxyprogesterone (Table 5).

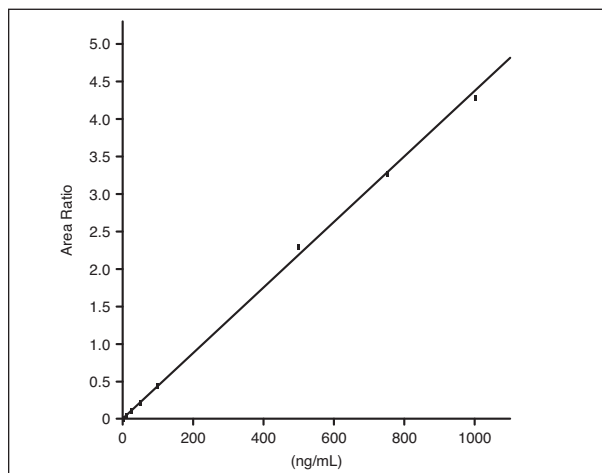


Figure 5. Linear dynamic range of 1 to 1000 ng/mL with an r^2 of 0.9992 for 11- α hydroxyprogesterone standards

Compound	n	QC Level	Accuracy	Precision
Corticosterone	6	High	-2.52 %	1.3 %
	6	Low	-1.81 %	2.5 %
11- α hydroxyprogesterone	6	High	-3.83 %	2.0 %
	6	Low	1.50 %	3.4 %

Table 4. Summary of corticosterone and 11- α hydroxyprogesterone results

Standard	Corticosterone	11- α hydroxyprogesterone
S1 response (peak area)	1276	2490
Total carryover (peak area)	174	192
% of S1 response	13.6	7.7

Table 5. Summary of carryover results

Conclusion

SOLA SPE well plates and Accucore RP-MS can be successfully used to extract and quantify four steroids from urine. The results show good linearity, accuracy and precision for all compounds demonstrating the capability of both the SOLA well plate and the Accucore RP-MS column.

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