

# APPLICATIONS

## A Rapid Extraction of Corticosteroids from Urine Using a Strata® RP On-Line Solid Phase Extraction Column

Xianrong (Jenny) Wei, Matthew Brusius, and Sean Orłowicz  
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

### Overview

In this application note, corticosteroids are extracted from urine using on-line extraction with a Thermo Cohesive System run in TX mode. A 50 x 0.5 mm Strata RP solid phase extraction (SPE) column in conjunction with a 50 x 3.0 mm Kinetex® 2.6 µm Biphenyl analytical LC column results in a total runtime of less than six minutes including system equilibration. **Figure 1** displays the Aria® based software overview and **Table 1** provides the on-line SPE conditions and **Table 2** shows the LC methodology. **Table 3** displays the calculated assay recovery, showing that all nine compounds except for Triamcinolone (the most polar compound in the suite) provide recovery greater than 73% and in all cases, show relative standard deviation less than 12.1%. **Figure 2** is a representative TIC for a 50 ng/mL spike into urine.

### Materials

Standards were purchased from Cerilliant® (Round Rock, TX). Double charcoal stripped human serum was purchased from BioreclamationIVT® (Westbury, NY) All other reagents and chemicals were obtained from Sigma-Aldrich®.

### Experimental Conditions

#### Sample Pre-treatment:

Human urine is diluted 1:1 with 10 mM Ammonium acetate.

#### On-Line Solid Phase Extraction

**On-line SPE Column:** Strata RP  
**Dimensions:** 50 x 0.5 mm  
**Part No.:** 00B-S326-AF

#### LC-MS/MS Conditions

**LC Column:** Kinetex 2.6 µm Biphenyl  
**Dimensions:** 50 x 3.0 mm  
**Part No.:** 00B-4622-Y0  
**Guard Column:** SecurityGuard™ ULTRA Cartridges  
**Part No.:** AJ0-9208  
**Mobile Phase:** A: 10 mM Ammonium Acetate in Water  
B: 10 mM Ammonium Acetate in Methanol  
**Gradient:** See Table 2  
**Flow Rate:** See Table 2  
**Needle Wash 1:** Methanol/Water (50:50)  
**Needle Wash 2:** 0.1 % Formic acid in Water  
**Injection Volume:** 30 µL  
**Instrument:** Cohesive System run in TX Mode: Agilent® 1260 with Leap Technologies PAL® autosampler LX-2  
**Detector:** MS/MS (SCIEX 4000 QTRAP®) ESI+ @ 400 °C

**Figure 1.**  
Screenshot of Method from Aria Software

**Extraction Column Conditions (blue) Analytical Column Conditions (Pink)**

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	0.00	30	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	50.0	50.0
2	0.50	5	0.15	Step	30.0	70.0	-	-	====	in	0.25	Step	50.0	50.0
3	0.58	40	0.15	Step	10.0	90.0	-	-	T	in	0.25	Step	50.0	50.0
4	1.25	30	0.80	Step	10.0	90.0	-	-	====	in	0.40	Step	50.0	50.0
5	1.75	60	0.80	Step	-	100.0	-	-	====	in	0.40	Step	50.0	50.0
6	2.75	60	0.80	Step	-	100.0	-	-	====	out	0.40	Step	10.0	90.0
7	3.75	45	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	10.0	90.0
8	4.50	75	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	50.0	50.0
9	5.75	5	0.80	Step	70.0	30.0	-	-	====	out	0.40	Step	50.0	50.0

Flow Rate Unit in mL/min



# APPLICATIONS

**Table 1.**  
On-line SPE Conditions

Step	Time (min)	Flow Rate (mL/min)	0.1 % Formic acid in Water	0.1 % Formic acid in Methanol
1	0	0.8	70	30
2	0.5	0.15	30	70
3	0.58	0.15	10	90
4	1.25	0.8	10	90
5	1.75	0.8	0	100
6	2.75	0.8	0	100
7	3.75	0.8	70	30
8	4	0.8	70	30
9	5.75	0.8	70	30

**Table 2.**  
LC Conditions

Step	Time (min)	Flow Rate (mL/min)	0.1 % Formic acid in Water	0.1 % Formic acid in Methanol
1	0	0.4	50	50
2	0.5	0.25	50	50
3	0.58	0.25	50	50
4	1.25	0.4	50	50
5	1.75	0.4	50	50
6	2.75	0.4	10	90
7	3.75	0.4	10	90
8	4.5	0.4	50	50
9	5.75	0.4	50	50

**Table 3.**  
Assay Recovery Values

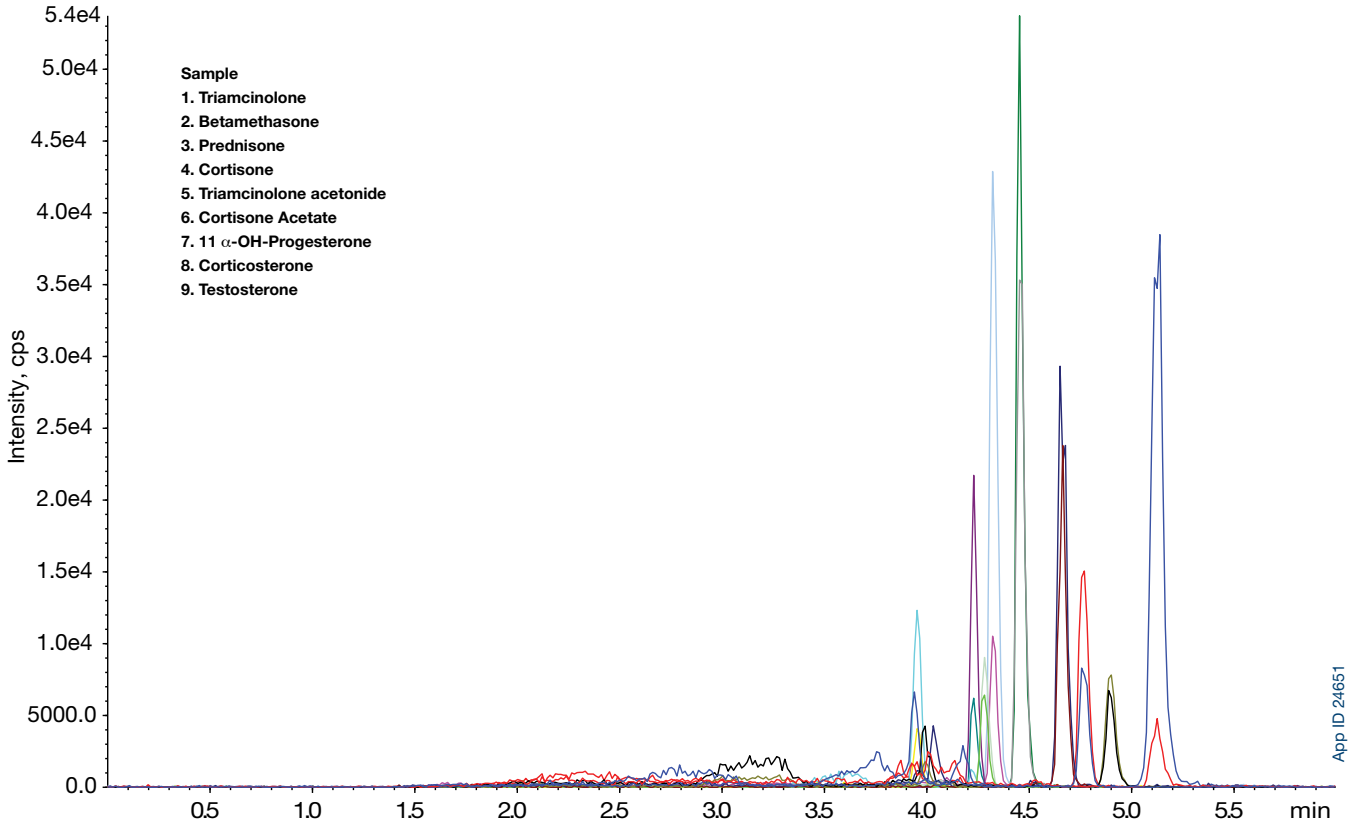
Analyte	% Recovery	% CV (n=6)
11- $\alpha$ -OH Progesterone	92.5	5.54
Triamcinolone Acetonide	93.7	12
Cortisone*	130	7.94
Betamethasone	73.4	4.82
Triamcinolone	50.3	8.25
Prednisone	84	4.28
Cortisone Acetate	91.4	10
Corticosterone	87.5	3.17
Testosterone	84	5.99

Assay Recovery = Analytes in Matrix / Analytes in Neat solution with TX mode

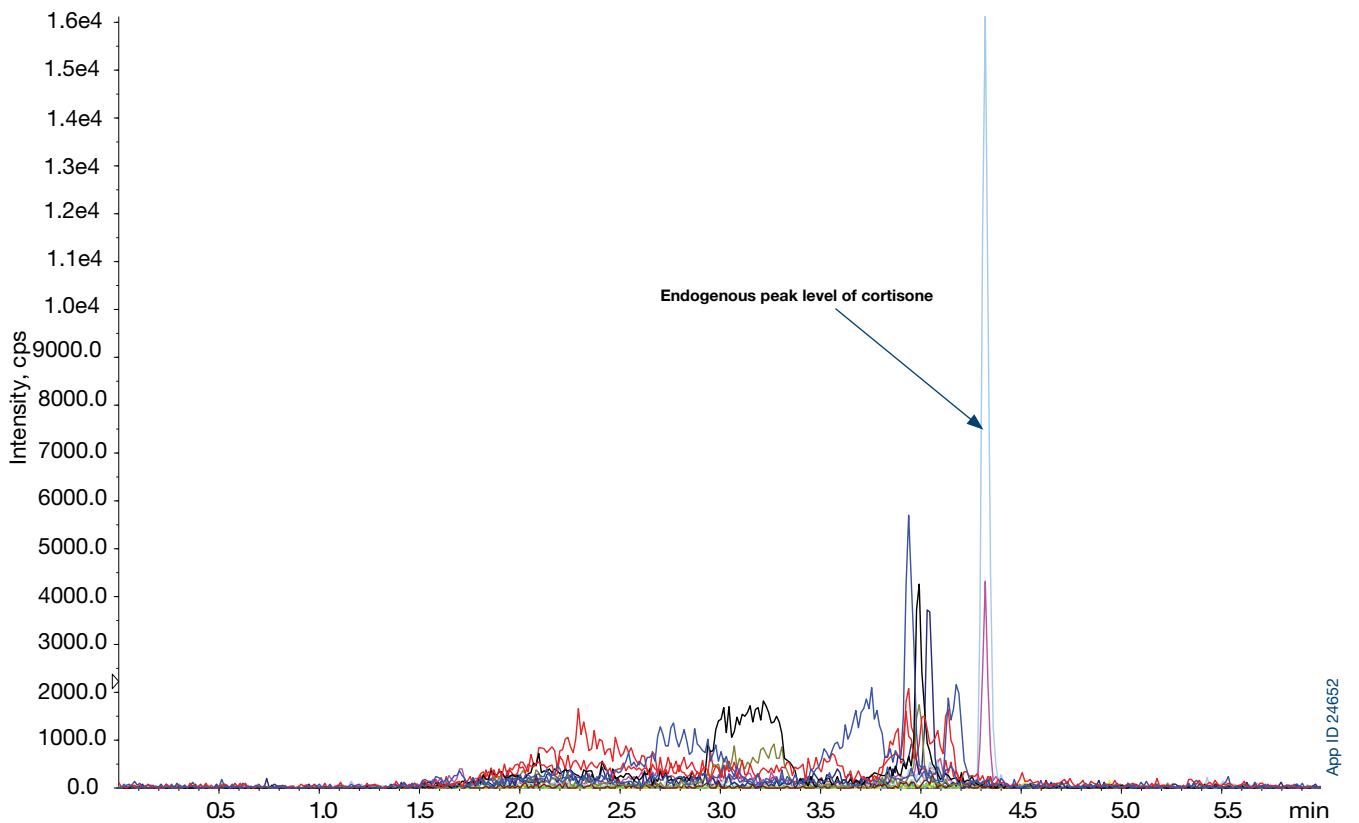
\*Blank urine contains endogenous level of cortisone

# APPLICATIONS

**Figure 2.**  
Representative of a Chromatogram of Human Urine at 50 ng/mL



**Figure 3.**  
Representative of a Chromatogram of Blank Human Urine



# APPLICATIONS

## Ordering Information Strata® On-Line SPE

On-Line Extraction Columns (mm)			
Phase	50 x 0.5	50 x 1.0	20 x 2.1
Strata RP	00B-S326-AF	00B-S326-AO	00M-S326-AN

## Kinetex® Core Shell LC Columns

Kinetex 2.6 µm MidBore™ Columns (mm)			SecurityGuard™ ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	150 x 3.0
Biphenyl	00B-4622-YO	00D-4622-YO	00F-4622-YO
			3/pk AJ0-9208 for 3.0 mm ID

† SecurityGuard ULTRA Cartridges required holder, Part No.: AJ0-9000.

Other particle sizes and dimensions are available. Visit [www.phenomenex.com/Kinetex](http://www.phenomenex.com/Kinetex) for a complete list of available columns.

### Australia

t: +61 (0)2-9428-6444  
f: +61 (0)2-9428-6445  
[auinfo@phenomenex.com](mailto:auinfo@phenomenex.com)

### Austria

t: +43 (0)1-319-1301  
f: +43 (0)1-319-1300  
[anfrage@phenomenex.com](mailto:anfrage@phenomenex.com)

### Belgium

t: +32 (0)2 503 4015 (French)  
t: +32 (0)2 511 8666 (Dutch)  
f: +31 (0)30-2383749  
[beinfo@phenomenex.com](mailto:beinfo@phenomenex.com)

### Canada

t: +1 (800) 543-3681  
f: +1 (310) 328-7768  
[info@phenomenex.com](mailto:info@phenomenex.com)

### China

t: +86 400-606-8099  
f: +86 (0)22 2532-1033  
[cninfo@phenomenex.com](mailto:cninfo@phenomenex.com)

### Denmark

t: +45 4824 8048  
f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### Finland

t: +358 (0)9 4789 0063  
f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### France

t: +33 (0)1 30 09 21 10  
f: +33 (0)1 30 09 21 11  
[franceinfo@phenomenex.com](mailto:franceinfo@phenomenex.com)

### Germany

t: +49 (0)6021-58830-0  
f: +49 (0)6021-58830-11  
[anfrage@phenomenex.com](mailto:anfrage@phenomenex.com)

### India

t: +91 (0)40-3012 2400  
f: +91 (0)40-3012 2411  
[indiainfo@phenomenex.com](mailto:indiainfo@phenomenex.com)

### Ireland

t: +353 (0)1 247 5405  
f: +44 1625-501796  
[eireinfo@phenomenex.com](mailto:eireinfo@phenomenex.com)

### Italy

t: +39 051 6327511  
f: +39 051 6327555  
[italiainfo@phenomenex.com](mailto:italiainfo@phenomenex.com)

### Luxembourg

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
[nlinfo@phenomenex.com](mailto:nlinfo@phenomenex.com)

### Mexico

t: 01-800-844-5226  
f: 001-310-328-7768  
[tecnicomx@phenomenex.com](mailto:tecnicomx@phenomenex.com)

### The Netherlands

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
[nlinfo@phenomenex.com](mailto:nlinfo@phenomenex.com)

### New Zealand

t: +64 (0)9-4780951  
f: +64 (0)9-4780952  
[nzinfo@phenomenex.com](mailto:nzinfo@phenomenex.com)

### Norway

t: +47 810 02 005  
f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### Portugal

t: +351 221 450 488  
f: +34 91-413-2290  
[ptinfo@phenomenex.com](mailto:ptinfo@phenomenex.com)

### Spain

t: +34 91-413-8613  
f: +34 91-413-2290  
[espinfo@phenomenex.com](mailto:espinfo@phenomenex.com)

### Sweden

t: +46 (0)8 611 6950  
f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### Switzerland

t: +41 61 692 20 20  
f: +41 61 692 20 22  
[swissinfo@phenomenex.com](mailto:swissinfo@phenomenex.com)

### United Kingdom

t: +44 (0)1625-501367  
f: +44 (0)1625-501796  
[ukinfo@phenomenex.com](mailto:ukinfo@phenomenex.com)

### USA

t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
[info@phenomenex.com](mailto:info@phenomenex.com)

### All other countries Corporate Office USA

t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
[info@phenomenex.com](mailto:info@phenomenex.com)



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

#### Terms and Conditions

Subject to Phenomenex Standard Terms & Conditions, which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

#### Trademarks

Strata and Kinetex are registered trademarks and MidBore and SecurityGuard are trademarks of Phenomenex. Cerilliant is a registered trademark of Cerilliant Corporation. BioreclamationIVT is a registered trademark of Bioreclamation-IVT Holdings, LLC. Sigma-Aldrich is a registered trademark of Sigma-Aldrich Co., LLC. Aria is a registered trademark of Thermo Fisher Scientific. Agilent is registered trademark of Agilent Technologies, Inc. QTRAP is a registered trademark of AB SCIEX Pte. Ltd. AB SCIEX is being used under license. PAL is a registered trademark of CTC Analytics AG.

#### Disclaimer

Phenomenex is not affiliated with Cerilliant, BioreclamationIVT, Sigma-Aldrich, ThermoFisher Scientific, or Agilent Technologies.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2018 Phenomenex, Inc. All rights reserved.

## www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at [international@phenomenex.com](mailto:international@phenomenex.com)