

APPLICATIONS

Automated Extraction of Isomeric Cortisone and Prednisolone from Plasma using Novum[®] Simplified Liquid Extraction (SLE) and Analysis by a Kinetex[®] Core-Shell Biphenyl HPLC/UHPLC Column

Shahana Huq, Seyed Sadjadi, and Matt Brusius
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

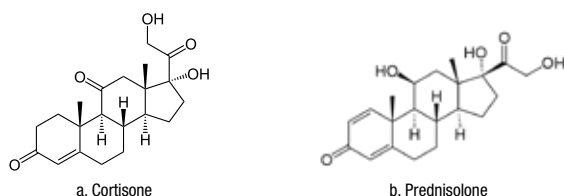


Matt Brusius
Product Manager,
Sample Preparation
Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.

Introduction

There is a need for more sensitive analytical tools to detect and confirm corticosteroids drugs. Chromatographic separation is essential for detection and confirmation of corticosteroids because cortisone and prednisolone are isomeric compounds (**Figure 1**). A simple automated extraction method using a Tecan Freedom EVO[®] 100 liquid handler and Phenomenex's Novum Simplified Liquid Extraction (SLE) 96-well plate is employed to extract corticosteroids from plasma samples. A Kinetex 2.6 μm , 50 x 3 mm core-shell Biphenyl HPLC/UHPLC column is used to successfully separate these two compounds which will be beneficial for detection and confirmation of the two isomers.

Figure 1.
Structure of cortisone (a) and prednisolone (b)



Experimental Conditions

Extraction Procedure

Sample pre-treatment

- Dilute 150 μL of human plasma (spiked with 25 ng/mL and 125 ng/mL of cortisone and prednisolone respectively) with 150 μL of 50 mM sodium phosphate dibasic heptahydrate, pH unadjusted. Mix briefly (3-5 sec).

Sample loading

- Load the sample from pre-treatment step above onto the Novum SLE plate (Part No. 8E-S138-FGA) and apply a short and gentle pulse of vacuum (~5-10" of Hg for 20 secs) until the sample has completely entered the media.
- Wait for 5 minutes.

Elution

- Dispense 1000 μL of ethyl acetate onto the Novum SLE media and allow the solvent to elute by gravity (~ 5 min elution time) and collect the eluant.
- Apply vacuum at 5" of Hg for 45 secs to complete the extraction.

NOTE: Prolonged application of vacuum will result in elution of plasma out of the Novum SLE media and into the final extracted solvent.

Dry down

- Evaporate the final extract to complete dryness under slow stream of N_2 at 40 $^\circ\text{C}$.
- Reconstitute the dry residue in 150 μL of initial mobile phase fortified with cortisol-D4.

HPLC Conditions

Column: Kinetex 2.6 μm Biphenyl
Dimensions: 50 x 3.0 mm
Part No.: 00B-4622-Y0
Mobile Phase: A: 10 mM Ammonium acetate in Water
 B: 10 mM Ammonium acetate in Methanol

Gradient:	Time (min)	% B
	0	50
	2	95
	3.1	95
	3.11	50
	5	50

Flow Rate: 0.45 mL/min
Inj. Volume: 10 μL
Temperature: Ambient
Detection: MS/MS, API 5000[™] (SCIEX), ESI+

Table 1.
MRM transitions & retention times for cortisone and prednisolone

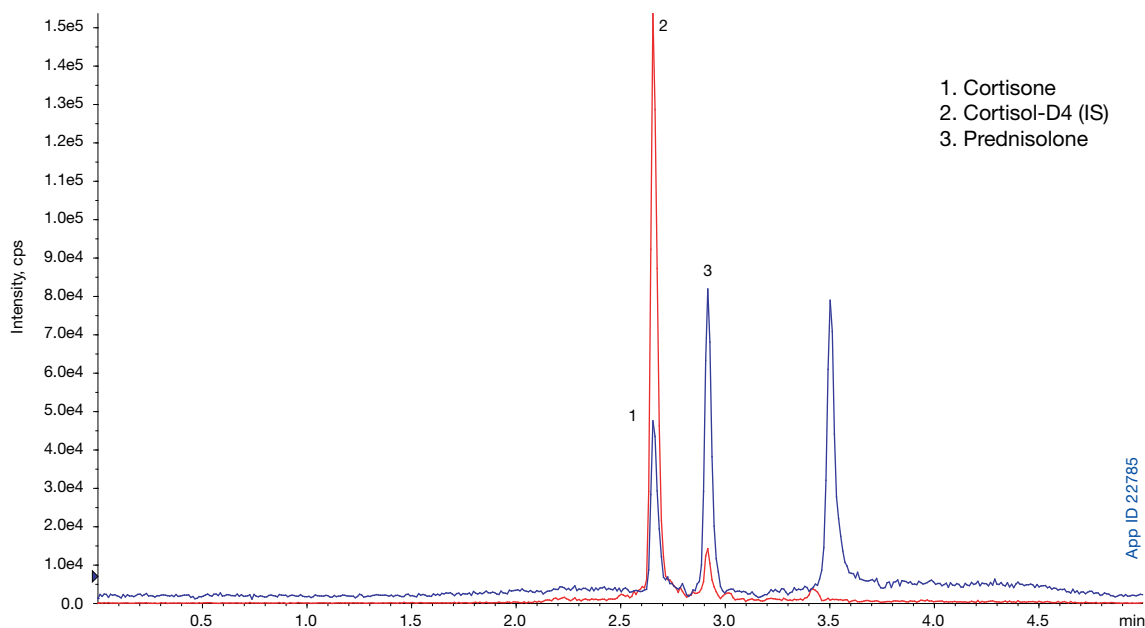
Peak #	Analyte	RT, min	Q1, Da	Q3, Da	CE
1	Cortisone	2.66	361.1	121.2	28
			361.1	163.2	
2	Cortisol-D4 (IS)	2.69	367.1	121.1	28
3	Prednisolone	2.92	361.1	121.2	28
			361.1	163.2	

Table 2.
Recovery of cortisone and prednisolone in human plasma after extraction with Novum SLE

Analyte	% Absolute Recovery	%CV (N=16)
Cortisone	99	3.6
Prednisolone	95	5.4

APPLICATIONS

Figure 2.
Separation of isomers cortisone and prednisolone



Results and Discussion

Prednisolone and Cortisone were extracted from plasma samples using Novum[®] SLE 96-well plates. In an effort to streamline the process, the extraction was automated using a Tecan Freedom EVO[®] 100 liquid handler. The Novum SLE protocol followed a simple load, wait, elute procedure which allowed us to process 96 samples in less than 15 minutes. By automating the procedure, we ensured that the method was consistent and reproducible because there is no chance for human error.

The extracted sample was then analyzed by LC-MS/MS using a Kinetex core-shell Biphenyl column (**Figure 2**). Separation of the two isomeric compounds was achieved, allowing us to accurately detect and confirm the presence of both cortisone and prednisolone. Recoveries for cortisone and prednisolone were 99 % and 95 % with CV values of 3.6 and 5.4 (N=16), respectively (**Table 2**). This indicates that our extraction method was both acceptable and accurate.

Conclusions

The automated extraction of cortisone and prednisolone from human plasma using Novum SLE 96-well plates along with Tecan's Freedom EVO 100 liquid handler yields quantitative recovery of both cortisone and prednisolone. The analytical method using a Kinetex core-shell Biphenyl HPLC/UHPLC column with MS/MS detection shows good selectivity for the two isomeric compounds.

APPLICATIONS

Ordering Information

Novum[®] Simplified Liquid Extraction (SLE)

Part No.	Description	Unit/Box
8E-S138-FGA	Novum SLE 96-Well Plate MINI	1/Box
8E-S138-5GA	Novum SLE 96-Well Plate MAX	1/Box

Accessories

Collection Plates (deep well, polypropylene)

AH0-7192	96-Well Collection Plate, 350 µL/well	50/pk
AH0-7193	96-Well Collection Plate, 1 mL/well	50/pk
AH0-7194	96-Well Collection Plate, 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH0-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk

Sealing Mats

AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-7362	Sealing Tape Pad	10/pk

Vacuum Manifold

AH0-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea
----------	---	----

Ordering Information

Kinetex[®] HPLC/UHPLC Columns

5 µm Minibore Columns (mm)			SecurityGuard [™] ULTRA Cartridges [‡]
Phase	50 x 2.1	100 x 2.1	3/pk
Biphenyl	00B-4627-AN	00D-4627-AN	AJ0-9209 for 2.1 mm ID

5 µm MidBore [™] Columns (mm)			SecurityGuard [™] ULTRA Cartridges [‡]
Phase	50 x 3.0	100 x 3.0	3/pk
Biphenyl	00B-4627-Y0	00D-4627-Y0	AJ0-9208 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard [™] ULTRA Cartridges [‡]
Phase	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207 for 4.6 mm ID

2.6 µm Minibore Columns (mm)					SecurityGuard [™] ULTRA Cartridges [‡]
Phase	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJ0-9209 for 2.1 mm ID

2.6 µm MidBore Columns (mm)				SecurityGuard [™] ULTRA Cartridges [‡]
Phase	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208 for 3.0 mm ID

2.6 µm Analytical Columns (mm)				SecurityGuard [™] ULTRA Cartridges [‡]
Phase	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Biphenyl	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJ0-9207 for 4.6 mm ID

1.7 µm Minibore Columns (mm)				SecurityGuard [™] ULTRA Cartridges [‡]
Phase	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209 for 2.1 mm ID

[‡] SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000



APPLICATIONS

Australia

t: +61 (0)2-9428-6444
 auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
 anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
 t: +32 (0)2 511 8666 (Dutch)
 beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
 info@phenomenex.com

China

t: +86 400-606-8099
 cninfo@phenomenex.com

Denmark

t: +45 4824 8048
 nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
 nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
 franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
 anfrage@phenomenex.com

India

t: +91 (0)40-3012 2400
 indiainfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
 eireinfo@phenomenex.com

Italy

t: +39 051 6327511
 italiainfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
 nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
 tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
 nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
 nzinfo@phenomenex.com

Norway

t: +47 810 02 005
 nordicinfo@phenomenex.com

Poland

t: +48 (12) 881 0121
 pl-info@phenomenex.com

Portugal

t: +351 221 450 488
 ptinfo@phenomenex.com

Singapore

t: +65 800-852-3944
 sginfo@phenomenex.com

Spain

t: +34 91-413-8613
 espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
 nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
 swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
 twinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
 ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
 info@phenomenex.com

**☎ All other countries/regions
 Corporate Office USA**

t: +1 (310) 212-0555
 info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com


Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks

Kinetex and Novum are registered trademarks and BE-HAPPY, MidBore, and SecurityGuard are trademarks of Phenomenex. API 5000 is a trademark of AB SCIEX Pte. Ltd. AB SCIEX is being used under license. Freedom EVO is a registered trademark of Tecan Group Ltd.
 Novum is patent pending.

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

© 2020 Phenomenex, Inc. All rights reserved.