Extraction of Total Cortisol from Human Plasma Using Novum™ Simplified Liquid Extraction (SLE) 96-Well Plates

Shahana Huq
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Abstract

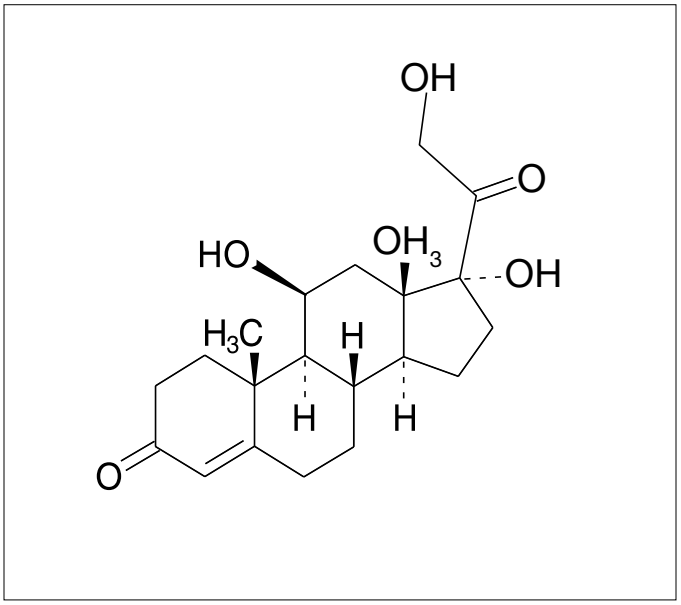
Existing methods for cortisol analysis are very diverse. This study focuses on developing an optimized sample preparation procedure for cortisol from plasma. The evaluation resulted in a rapid, clean, and reproducible method using Novum Simplified Liquid Extraction (SLE) 96-well plates.

Introduction

Cortisol or hydrocortisone is a corticosteroid secreted by the adrenal cortex. Cortisol is synthesized from cholesterol and may be found in the blood bound to globulin or in free-form. Cortisol has an anti-inflammatory effect and aids in carbohydrate metabolism, renal function, and the promotion of gluconeogenesis. Measurement of plasma cortisol levels is useful when studying the functions of the adrenal cortex, including Cushing's syndrome (hypercortisolism), Addison's disease (hypocortisolism), and adrenal tumors. Abnormal cortisol levels may be linked to prostate cancer, depression, and schizophrenia.

The aim of this work is to introduce a fast and effective plasma cortisol extraction procedure using Novum SLE which results in a clean extract with high recovery. To determine the most effective procedure, two different extraction solvents were examined; dichloromethane (methylene chloride or DCM) and a more polar elution solvent, methyl tert-butyl ether (MTBE)/ethyl acetate (EtOAc) (50:50).

Figure 1. Structure of Cortisol



Analytes

1. Cortisol
2. Cortisol-D4 as Internal Standard

APPLICATIONS

Experimental Conditions

Extraction Procedure

Sample Pre-treatment

- Dilute 200 µL of human plasma (spiked with cortisone at 25 ng/mL) with 200 µL of 50 mM sodium phosphate dibasic heptahydrate, pH unadjusted. Vortex briefly (3-5 sec).

Sample Loading

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

Elution

- Dispense 900 µL of elution solvent onto the Novum SLE media and allow the solvent to elute by gravity (~ 5 min elution time) and collect the eluant.
- Repeat with another 900 µL of elution solvent and collect the eluant.
- Apply vacuum at 5" of Hg for 20-30 secs to complete the extraction.

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

Dry Down

- Evaporate the final extract to complete dryness under a slow stream of N_2 at 40 °C.
- Reconstitute the dry residue in 200 µL of initial mobile phase fortified with cortisol-D4.

HPLC Conditions

Column: Kinetex® 2.6 µm Biphenyl
Dimensions: 50 x 2.1 mm
Part No.: 00B-4622-AN
Mobile Phase: A: 10 mM Ammonium acetate in Water
B: 10 mM Ammonium acetate in Methanol
Gradient:

Time (min)	% B
0.00	50
2.00	95
3.10	50
5.00	50

Temperature: Ambient
Detection: MS/MS, API 5000™ (AB SCIEX), ESI+
Injection : 10 µL

MS/MS Conditions

CAD: 7.00
CUR: 25.00
GS1: 50.00
GS2: 50.00
IS: 5000.00
TEM: 600.00

Analyte	RT, min	Q1, Da	Q3, Da	CE
Cortisol	2.13	363.1	120.0 309.1	27 22
Cortisol-D4	2.13	367.1	121.1	28

Figure 2. Representative chromatogram of plasma cortisol (25 ng/mL) extracted by a more polar solvent combination (MTBE/EtOAc (50:50)) (notice the region enclosed in red circle).

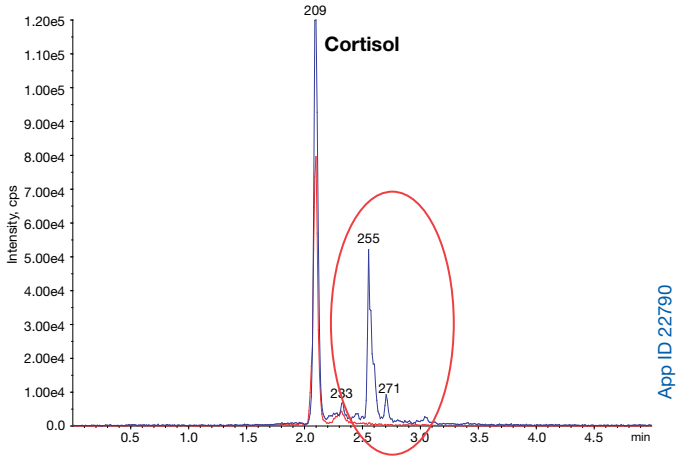


Figure 3. Representative chromatogram of plasma cortisol (25 ng/mL) extraction using DCM as an extraction solvent (notice the region enclosed in red circle).

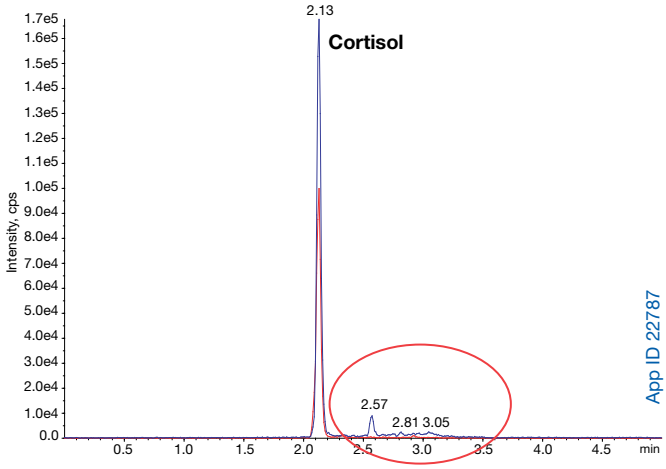


Table 1. Absolute recovery of cortisol using the optimized Novum SLE procedure (DCM elution solvent)

Analyte	% Absolute Recovery	%CV (N=8)
Cortisol	79%	6.8

Results and Discussion

Cortisol was extracted from plasma using Novum SLE 96-well plates. In order to determine the most effective extraction method in terms of cleanup and recovery, two different elution solvents were examined. The first elution solvent, a more polar combination of MTBE/EtOAc (50:50), resulted in acceptable Cortisol recoveries however the resulting chromatogram (**Figure 2**) revealed that matrix contaminants were also eluted from the the Novum SLE sorbent. The second elution solvent, DCM, resulted in a much cleaner extract (**Figure 3**) and an absolute recovery of 79 % (**Table 1**).

Conclusion

The Novum SLE extraction with DCM shows superior cleanliness as it reduces the late eluting peaks in the chromatogram. The prescribed procedure provides reproducible recoveries of cortisol from human plasma.

APPLICATIONS

Ordering Information

Novum™ Simplified Liquid Extraction (SLE)

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

Accessories

Collection Plates (deep well, polypropylene)		Unit
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

Kinetex® Core-Shell HPLC/UHPLC Columns

5 µm Minibore Columns (mm)

Phase	50 x 2.1	100 x 2.1	SecurityGuard™ ULTRA Cartridges‡ 3/pk
Biphenyl	00B-4627-AN	00D-4627-AN	AJ0-9209 for 2.1 mm ID

5 µm MidBore™ Columns (mm)

Phase	50 x 3.0	100 x 3.0	SecurityGuard ULTRA Cartridges‡ 3/pk
Biphenyl	00B-4627-Y0	00D-4627-Y0	AJ0-9208 for 3.0 mm ID

5 µm Analytical Columns (mm)

Phase	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard ULTRA Cartridges‡ 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)

Phase	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges‡ 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)

Phase	50 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard ULTRA Cartridges‡ 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)

Phase	50 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard ULTRA Cartridges‡ 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)

Phase	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges‡ 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

guarantee

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

APPLICATIONS

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

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
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

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

China
t: +86 (0)20 2282-6668
f: +86 (0)20 2809-8130
chinainfo@phenomenex.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

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

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

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

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

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

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

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

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

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

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

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

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

www.phenomenex.com

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

TN27271014_W