# 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

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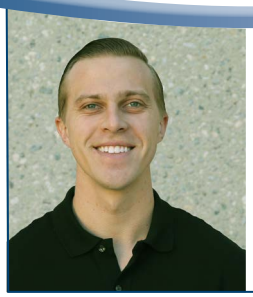
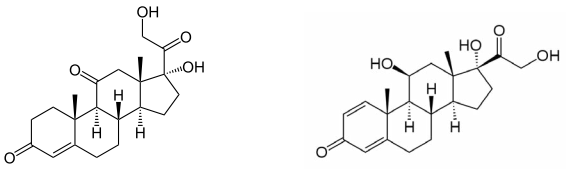


**Matt Brusius**
**Product Manager,**
**Sample Preparation**
*Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.*

## Introduction

Corticosteroids exhibit anti-inflammatory properties and are widely abused within the sports industry. Hence there is a need for more sensitive analytical tools to detect and confirm these classes of drugs. Chromatographic separation is essential for detection and confirmation of corticosteroids because cortisone and prednisolone are isomeric compounds. 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)




a. Cortisone b. Prednisolone

## 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 MINI 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 °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 |
|---|---|
| 0.00 | 50 |
| 2.00 | 95 |
| 3.10 | 95 |
| 3.11 | 50 |
| 5.00 | 50 |

**Flow Rate:** 0.45 mL/min
**Inj. Volume:** 10 µL
**Temperature:** Ambient
**Detection:** MS/MS, API 5000™ (AB 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<br>361.1 | 121.2<br>163.2 | 28 |
| 2 | Cortisol-D4 (IS) | 2.69 | 367.1 | 121.1 | 28 |
| 3 | Prednisolone | 2.92 | 361.1<br>361.1 | 121.2<br>163.2 | 28 |

**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 |

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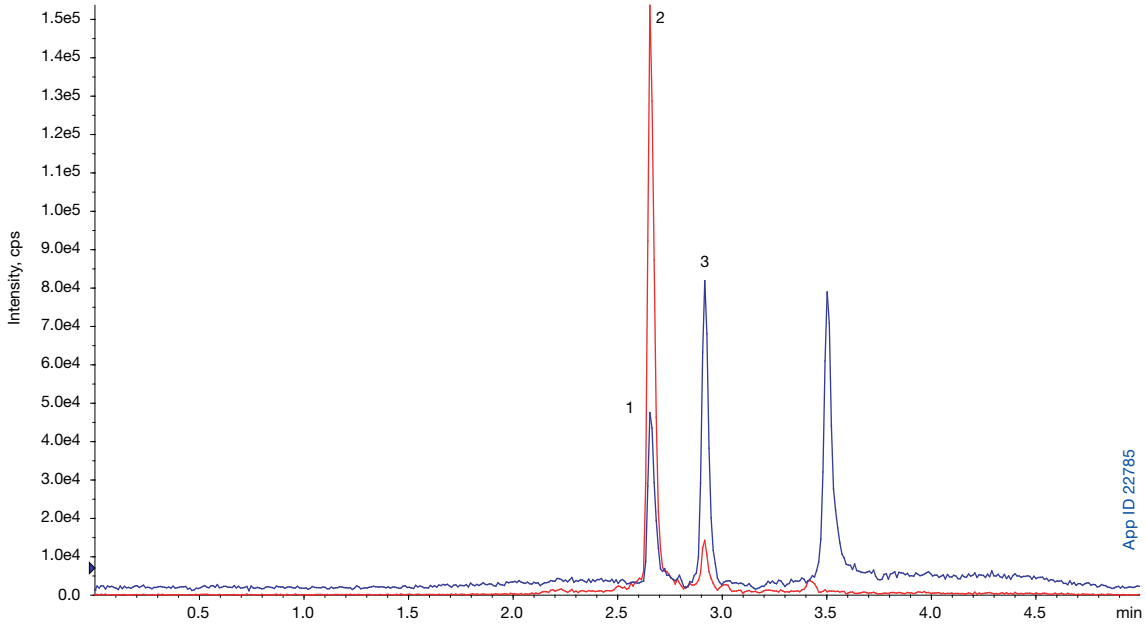


## Results and Discussion

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.

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## 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)** | | |
| 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) | | | 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

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.

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