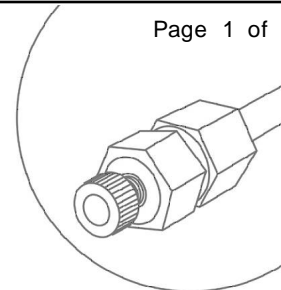


## Steroids on Strata<sup>™</sup> X

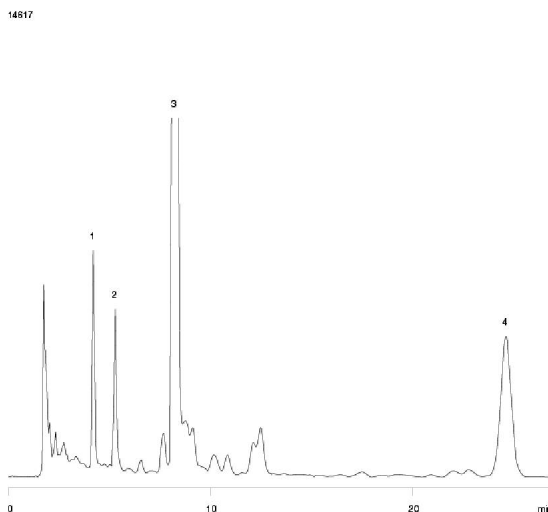
Column: Synergi 4 $\mu$  Max-RP 80Å  
 Dimensions: 150 x 4.6 mm ID  
 Order No: 00F-4337-E0  
 Elution Type: Isocratic  
 Eluent A: 20 mM Potassium Phosphate, pH 7 Pct. A: 35  
 Eluent B: Methanol Pct. B: 65  
 Flow Rate: 1 mL/min  
 Col. Temp.: ambient  
 Detection: UV-Vis Abs. - Variable Wave. @ 254 nm (ambient)  
 Solid Phase Extraction (SPE): strata-X 33 $\mu$ m Polymeric Reversed Phase, 30 mg / 1 mL, Tube, 100/Pk  
 Order No.: 8B-S100-TAK  
 See Sample Preparation report for full details of the extraction procedure



**Products used in this Application:**

**synergi**<sup>™</sup>

**strata**X



**ANALYTES:**

- 1 Prednisolone
- 2 Betamethasone
- 3 Butyl paraben (extrenal standard)
- 4 Betamethasone Valerate

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# Sample Preparation Details

for HPLC Application ID No.: 14617

## Steroids on Strata™ X

Page 1 of 1

### PRODUCT DESCRIPTION:

strata-X 33µm Polymeric Reversed Phase  
30 mg / 1 mL, Tube, 100/Pk  
Order No.: 8B-S100-TAK

### SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 30 mg bed mass.  
The solvent volumes will need to be adjusted for a smaller or larger bed mass.

#### Condition:

1. Methanol 1.0 mL
2. Water 1.0 mL

#### Load:

1. Prednisolone (1ug/ml)
2. Betamethasone (1ug/ml)
3. Butyl paraben (I.S.) (0.1 mg/ml)
4. Betamethasone Valerate (4ug/ml)

#### Wash:

1. Methanol:Water 1.0 mL

#### Dry:

1 min @ 10 in. Hg

#### Elute:

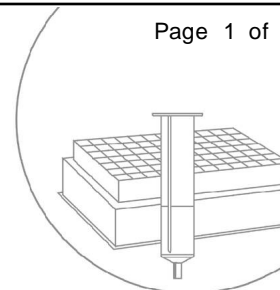
1. Methanol 1.0 mL

#### Final Prep and Analysis:

Add 25 mL of 0.1 mg/ml butyl paraben (I.S.). 40 °C under N<sub>2</sub> stream and reconstitute in 200 µL Acetonitrile. Inject: 5 µL on HPLC / UV-Vis Abs. - Variable Wave. @ 254 nm (ambient)

#### ANALYTES:

	Spiked Conc. (µg/mL)	Log P	pKa	% Rec	%RSD (n=4)
1 Prednisolone	1			94	3.7
2 Betamethasone	1			99	3.5
3 Butyl paraben (extrenal standard)	0.1				
4 Betamethasone Valerate	4			95	3.9

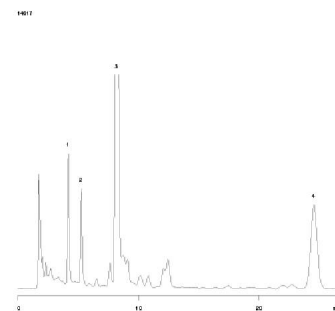


Products used in this Application:

**synergi**™

**strata**X

### HPLC CHROMATOGRAM



Synergi 4µ Max-RP 80Å  
150 x 4.6 mm ID  
Order No.: 00F-4337-E0

See HPLC report for full conditions and separation details.

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your Phenomenex Representative for assistance in method development and optimization techniques.

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