

APPLICATIONS

Extraction and Analysis of Budesonide and 16 α -Hydroxyprednisolone in Dog Plasma Using Strata[®]-X and Kinetex[®] 2.6 μ m C18

Matthew Trass and Ramkumar Dhandapani
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Ramkumar Dhandapani
Technical Specialist
Ramkumar loves to write poems, and to watch & read Shakespeare's plays.



Introduction

Budesonide is a nonsystemic corticosteroid with numerous applications for humans including the treatment of asthma and inflammatory bowel diseases. It is also commonly prescribed as veterinary medication predominantly used for canine inflammatory disease. Despite its widespread use, knowledge is lacking regarding the pharmacokinetics in canine veterinary medicine. In order to study budesonide and its impact on dogs, an effective and robust analytical method is necessary. Here we present an LC/MS/MS method for the determination of budesonide and its primary metabolite, 16 α -hydroxyprednisolone, in dog plasma. This method uses utilizes Strata-X Solid Phase Extraction (SPE) of a plasma sample, followed by LC/MS/MS analysis using a Kinetex 2.6 μ m C18 analytical HPLC column.

Experimental Conditions

Materials and Methods

Budesonide, 250 mg (Sigma Aldrich[®], Part No.: B7777)

16 α -Hydroxyprednisolone (SynThink Research Chemicals, Part No.: SA14103)

Betamethasone (BET), 100mg (Sigma Aldrich, Part No.: 34166)
0.2 μ m Filtered Canine Plasma, Disodium EDTA/Female (BioreclamationIVT)

SPE Conditions

Cartridge:	Strata-X 30mg /1mL
Part No.:	8B-S100-TAK
Pretreatment:	1 mL plasma/reagent water (1:1) and spike with BET internal standard
Condition:	1 mL Methanol
Wash:	1 mL Water
Load:	1 mL pretreated plasma samples
Wash:	3 mL Methanol/Water (5:95)
Dry:	Under 10 Hg for 2 min
Elute:	1.5 mL Methanol
Evaporate:	To dryness under nitrogen at 35 °C
Reconstitute:	Add 100 μ L of Methanol/Water (25:75) to dried down sample

LC/MS/MS Conditions

Column:	Kinetex 2.6 μ m C18
Dimensions:	50 x 2.1 mm
Part No.:	00B-4462-AN
Mobile Phase:	A: 0.1% Formic acid B: 0.1% Formic acid in Methanol
Gradient:	Time (min) B (%)
	0 25
	3 95
	5 95
Flow Rate:	0.5 mL/min
Injection:	20 μ L
Temperature:	Ambient
Detection:	API 5000 [™] , ESI +ve (SCIEX)

MS conditions:

GS1:	55
GS2:	55
IS:	5000
Temp:	600

Table 1. MRM Transitions

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	DP	CE
16 α -Hydroxyprednisolone	377.2	226.4/147.5	50	105/110	31/36
Betamethasone	393.2	373.3/355.2	50	105/110	13/19
Budesonide	431.2	323.1/147.3	50	75/120	19/42

Figure 1. Plasma extract spiked with 2 ng/mL on a Kinetex 2.6 μ m C18

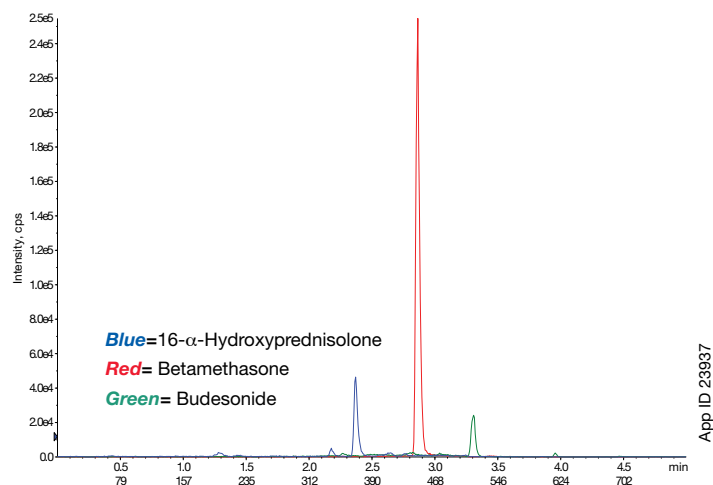


Figure 2. Zoomed baseline of blank plasma extract on a Kinetex 2.6 μ m C18

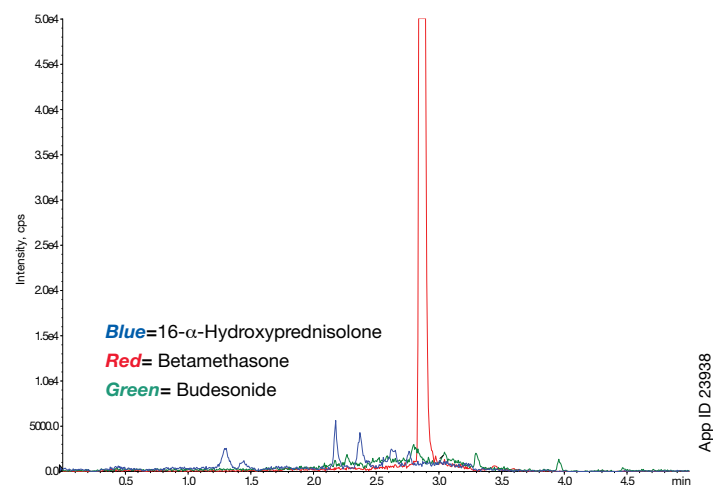


Figure 3. Hydrocyprednisolone plasma extracted calibration curve from 1.0-20 ng/mL, $r^2=0.9999$

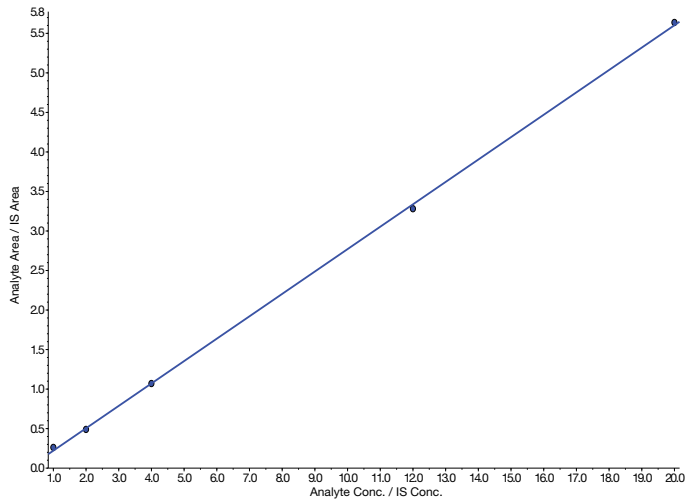
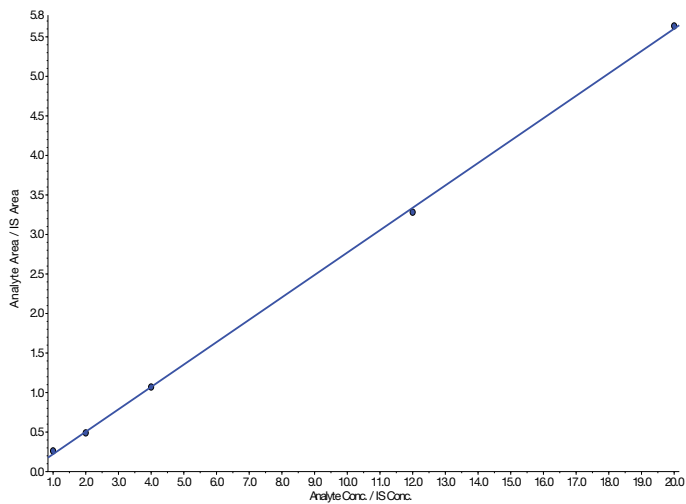


Figure 4. Budesonide plasma extracted calibration curve from 1.0-20 ng/mL, $r^2=0.9999$



Results

Presented here is an SPE method using Strata[®]-X to extract budesonide and its primary metabolite from plasma, followed by LC/MS/MS analysis. For chromatographic separation, a Kinetex[®] 2.6 μ m C18 column is used because of the high efficiency that core-shell columns provide as well as excellent peak-shape and resolution for budesonide, 16 α -hydrocyprednisolone and betamethasone (I.S.) as shown in **Figure 1**. Additionally, very little matrix interference is observed, resulting in easily quantifiable peaks at relatively low concentrations as shown in **Figure 2**. The optimized SPE procedure using Strata-X not only helped in cleaning up matrix interferences but also provided excellent linearity for 1-20 mg/mL of budesonide metabolites as presented in **Figure 3** and **4**.

Conclusion

The Kinetex 2.6 μ m C18 HPLC column displays symmetrical peak-shape and excellent resolution for the analysis of budesonide and its primary metabolite, 16 α -hydrocyprednisolone. By pretreating canine plasma samples with a simple and straight-forward Strata-X SPE protocol, the majority of the matrix interferences are removed. By cleaning up the sample, low detection limits are observed and down-time due to instrument maintenance is minimized.

References

1. Gazzotti T, Barbarossa A, Zironi E, Roncada P, Pietra M, Pagliuca G., An LC-MS/MS method for the determination of budesonide and 16 α -hydrocyprednisolone in dog plasma. ELSEVIER (2016). Volume 3: 139-143 (2006), 48-6172,525-529.6.

Ordering Information
Kinetex® Core-Shell HPLC/UHPLC Columns

5 µm Columns (mm)		SecurityGuard™ ULTRA Cartridges†					SecurityGuard ULTRA Cartridges†
Phases	50 x 2.1	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-AN	AJO-8782 for 2.1 mm ID	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJO-8768 for 4.6 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges†
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJO-8768 for 4.6 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges†
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
XB-C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJO-8775 for 3.0 mm ID

2.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJO-8782 for 2.1 mm ID





















1.7 µm MidBore Columns (mm)			SecurityGuard ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	3/pk
C18	00B-4475-Y0	00D-4475-Y0	AJO-8775 for 3.0 mm ID

1.7 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJO-8782 for 2.1 mm ID

1.3 µm Columns (mm)	
Phase	50 x 2.1
C18	00B-4515-AN

†SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000

Ordering Information
Strata®-X SPE

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S100-TAK**	1 mL (100/box)
	30 mg	8B-S100-TBJ	3 mL (50/box)
	60 mg	8B-S100-UBJ**	3 mL (50/box)
	100 mg	8B-S100-EBJ	3 mL (50/box)
	100 mg	8B-S100-ECH	6 mL (30/box)
	200 mg	8B-S100-FBJ	3 mL (50/box)
	200 mg	8B-S100-FCH	6 mL (30/box)
	500 mg	8B-S100-HBJ	3 mL (50/box)
	500 mg	8B-S100-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S100-HDG	12 mL (20/box)
	1 g	8B-S100-JDG	12 mL (20/box)
	1 g	8B-S100-JEG	20 mL (20/box)
	2 g	8B-S100-KEG	20 mL (20/box)
	5 g	8B-S100-LFF	60 mL (16/box)
Teflon® Tube			
	200 mg	8B-S100-FBJ-T	3 mL (50/box)
	200 mg	8B-S100-FDG-T	12 mL (20/box)
96-Well Plate			
	10 mg	8E-S100-AGB	2 Plates/Box
	30 mg	8E-S100-TGB	2 Plates/Box
	60 mg	8E-S100-UGB	2 Plates/Box
96-Well Microelution Plate			
	2 mg	8M-S100-4GA	ea

** Tab-less tubes available. Contact Phenomenex for details.

Ordering Information
Presston™ 100 Positive Pressure Manifold

Part No.	Description
AH0-9334	Presston 100 Positive Pressure Manifold, 96-Well Plate
AH0-9342	Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly
AH0-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly
AH0-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly

The Presston 100 96-Well Positive Pressure Manifold can also process 1, 3, and 6 mL tubes using the following adapter kits
Presston 100 Tube Adapter Kits (for AH0-9334)

Part No.	Description
AH0-9344	1 mL Tube Adapter Kit
AH0-9345	3 mL Tube Adapter Kit
AH0-9346	6 mL Tube Adapter Kit



WARRANTY Phenomenex warrants that for a period of 12 months following delivery, the Presston 100 Positive Pressure Manifold you have purchased will perform in accordance with the published specifications and will be free from defects in materials or workmanship. In the event that the Presston 100 Positive Pressure Manifold does not meet this warranty, Phenomenex will repair or replace defective parts. Please visit www.phenomenex.com/Presston for complete warranty information.

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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)22 2532-1032
f: +86 (0)22 2532-1033
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: 01-800-844-5226
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

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