Highly Selective and Sensitive Determination of Betamethasone-17,21-Dipropionate, Betamethasone-17-Propionate and Betamethasone by LC-MS/MS

Nadia Smith, Philippe Bélanger, François Samson-Thibault, Marie-Josée Marcoux, Marie-Claude Théberge, Nadine Boudreau and Ann Lévesque (inVentiv Health Clinique, Québec, Canada)

Introduction

Betamethasone dipropionate is a glucocorticoid steroid with anti-inflammatory and immunosuppressive abilities. It is applied as a topical cream, ointment, lotion or gel to treat itching and other minor skin conditions such as eczema. Betamethasone dipropionate is metabolized to betamethasone-17-propionate, betamethasone-21-propionate and betamethasone. The purpose of this work is to develop methods for the determination of betamethasone dipropionate, betamethasone-17-propionate and betamethasone in human plasma. Moreover, the assay had to be selective for betamethasone-21-propionate since it has the same mass as betamethasone-17-propionate.

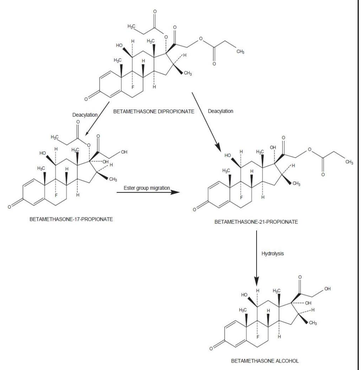
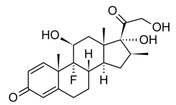
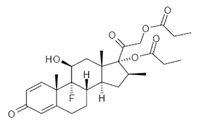


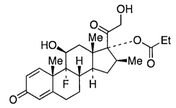
Figure 1. Betamethasone-17,21-dipropionate Metabolic Pathway



Betamethasone 392.46 amu



Betamethasone-17,21-dipropionate 504.58 amu



Betamethasone-17-propionate 448.52 amu

Method

Betamethasone-17, 21-dipropionate is extracted from human NaF/Na_2EDTA plasma using liquid-liquid extraction with 1-chlorobutane. Chromatography was performed with an ACE Excel 2 C18, 50 x 3 mm, 2 µm column. Betamethasone-17-propionate was extracted from human NaF/Na_2EDTA plasma by liquid-liquid extraction with a mixture of EtAc/chlorobutane. A Pursuit XRS Ultra C8 column was used for the analysis on the LC-MS/MS. Bethametasone was extracted from human NaF/Na_2EDTA plasma using an automated liquid-liquid extraction with a mixture of EtAc/hexanes. Chromatography was performed using an ACE Excel 2 C18, 50 X 3 mm, 2 µm column.

Extraction Procedure

	Betamethasone-17,21-dipropionate	Betamethasone-17-propionate	Betamethasone
Matrix	NaF/Na_2EDTA	NaF/Na_2EDTA	NaF/Na_2EDTA
Analytical Range	5-5000 pg/mL	5-5000 pg/mL	5-5000 pg/mL
Internal Standard	Betamethasone-17,21-dipropionate-d_5	Betamethasone-17-propionate-d_5	Dexamethasone-d_4
Sample Volume	0.200 mL	0.200 mL	0.250 mL
Extraction Type	Liquid-Liquid Extraction	Liquid-Liquid Extraction	Automated Liquid-Liquid Extraction
Concentration Factor	2	2	1

LC-MS/MS Analysis

	Betamethasone-17,21-dipropionate	Betamethasone-17-propionate	Betamethasone
Chromatographic Mode	Reverse Phase	Reverse Phase	Reverse Phase
Analytical Column	ACE Excel 2 C18	Pursuit XRS Ultra C8	ACE Excel 2 C18
Elution Mode	Isocratic	Isocratic	Isocratic
Mobile Phase A	MeOH/Water/Ammonium formate/Formic Acid	ACN/Water/Ammonium formate/Formic Acid	MeOH/Water/Ammonium formate/Formic Acid
Flow Rate	0.400 mL/min	0.400 mL/min	0.550 mL/min
Injection Volume	10 µL	15 µL	30 µL
Retention Time	1.59 min	2.20 min	1.55 min
Acquisition Time	3.50 min	4.50 min	4.00 min
Detector	API 5000	API 5000	API 5000
Source	TurboIonSpray	TurboIonSpray	TurboIonSpray
Ion Monitored	505→485	449→355	393→373

Results

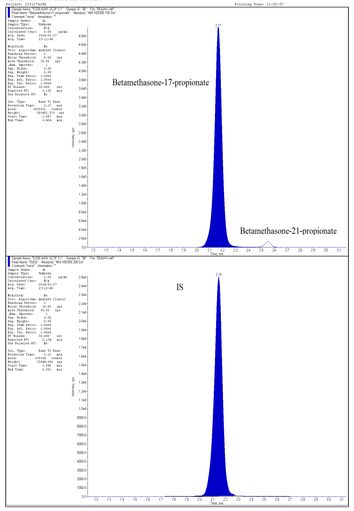


Figure 2. Representative Chromatogram of Betamethasone-17-Propionate (2.17 min) and Betamethasone-21-Propionate (2.56 min)

NaF/Na_2EDTA was used as anticoagulant in order to prevent hydrolysis of the propionate moieties in whole blood and plasma during sample collection, handling and storage. All analytes analytical methods were validated over the dynamic range of 5-5000 pg/mL. As betamethasone-21-propionate and betamethasone-17-propionate have the same molecular weight, they have to be separated using adequate chromatography conditions. This was achieved using the Pursuit XRS Ultra C8 100 x 2.0 mm, 2.8 µm column and a gradient of mobile phase composition. Retention times were of 2.17 and 2.56 minutes, for betamethasone-17-propionate and betamethasone-21-propionate, respectively **(Figure 2.)**. Other columns were tested unsuccessfully. Another issue was faced during method development regarding the stability of dipropionate and propionate. As they are ester forms of betamethasone, they can easily convert to betamethasone. For this reason, in addition to the use of NaF/Na_2EDTA anticoagulant, samples were kept at 4˚ C at all times. Hydrolysis of the propionate moieties were observed at room temperature when the incubation time was higher than 4 hours **(Table 1)**. Accuracy and precision were demonstrated for the three analytical methods and were below 5% and 8%, respectively **(Table 2)**. No matrix effect was observed, including from hemolyzed or hyperlipemic plasma. Interference evaluation of each analyte with the others was performed including stabilities in matrix under different stress conditions **(Table 3)**. However, the stability testings of the metabolites in the method for betamethasone-17,21-diprionate were not performed as their back-conversion into the parent is unlikely.

Table 1. Impact of Betamethasone-17,21-Dipropionate (5000 pg/mL) on the Concentration of Betamethasone in Plasma after Incubation at Room Temperature and 4°C

Conditions	Betamethasone Conc. (pg/mL)		% Change
	Comparison Sample (T=0)	Stability Sample	
11 hrs at RmT	15.48	19.53	26.2
11 hrs at 4°C	15.48	15.85	2.4
22 hrs at RmT	15.48	29.38	89.9
22 hrs at 4°C	15.48	16.29	5.3

Table 2. Inter-Run Accuracy and Precision

		LLQC (5 pg/mL)		QC1 (15 pg/mL)		QC2 (2500 pg/mL)		QC3 (3750 pg/mL)	
		Conc. (pg/mL)	% Bias	Conc. (pg/mL)	% Bias	Conc. (pg/mL)	% Bias	Conc. (pg/mL)	% Bias
Betamethasone-17,21-dipropionate	Mean	5.1	1.70	15.1	0.94	2474.9	-1.00	3795.5	1.21
	SD (±)	0.50		1.00		82.17		109.00	
	CV %	9.86		6.64		3.32		2.87	
Betamethasone-17-dipropionate	Mean	5.1	2.81	15.4	2.39	2570.5	2.82	3881.7	3.51
	SD (±)	0.71		1.25		90.60		173.21	
	CV %	13.75		8.05		3.52		4.46	
Betamethasone	Mean	4.9	-2.94	14	-6.47	2388.9	-4.44	3528.8	-5.90
	SD (±)	0.54		0.57		106.86		112.63	
	CV %	11.23		4.04		4.47		3.19	

Table 3. Impact of Betamethasone-17,21-dipropionate, Betamethasone-21-propionate and Betamethasone on Betamethasone-17-Propionate and Impact of Betamethasone-17,21-dipropionate, Betamethasone-21-propionate and Betamethasone-17-Propionate on Betamethasone under Different Stress Conditions

Parameters	Betamethasone-17-propionate		Betamethasone	
	Low QC (15 pg/mL)	High QC (3750 pg/mL)	Low QC (15 pg/mL)	High QC (3750 pg/mL)
Freeze/Thaw at -20°C	-1.9	-1.7	-6.9	-6.8
Freeze/Thaw at -80°C	-3.5	2.8	-6.7	-6.8
Short-Term at RmT (4 hrs)	-10.3	-7.3	-5.8	-8.6
Short-Term at 4°C (21 hrs)	-5.0	0.9	-2.9	-5.1
Post-Preparative at RmT (1)	11.2	7.3	-9.7	-6.7
Whole Blood at 4°C (2)	7.2	1.0	8.0	-5.2

(1) 70hrs for Betamethasone-17-propionate and 93hrs for Betamethasone
(2) 240 minutes for Betamethasone-17-propionate and 110 minutes for Betamethasone

Chromatography

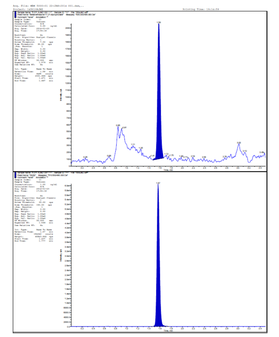


Figure 3. Representative Chromatogram of Lower Limit of Quantitation for Betamethasone-17,21-Dipropionate at 5 pg/mL

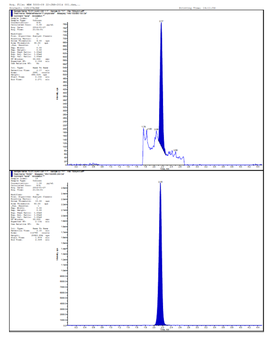


Figure 4. Representative Chromatogram of Lower Limit of Quantitation for Bethamethasone-17-Propionate at 5 pg/mL

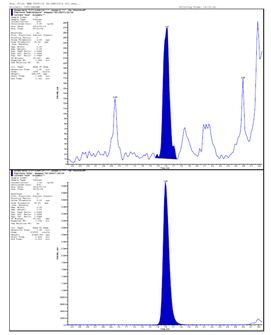


Figure 5. Representative Chromatogram of Lower Limit of Quantitation for Betamethasone at 5 pg/mL

Conclusion

The methods were validated as per the most recent regulatory guidelines. Moreover, interference with co-metabolites was assessed successfully. These methods were successfully applied for study sample analysis and were reproducible with reassay confirmation rates of nearly 100% in all cases.