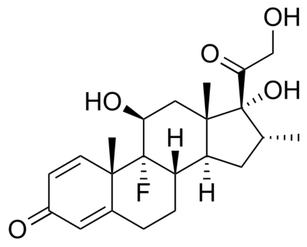
An Ultra-Sensitive Method for Femtogram Level Detection of Dexamethasone in Human Plasma using LC-MS/MS

Jason Bilodeau, François Viel, Nadine Boudreau and Ann Lévesque (inVentiv Health Clinical, Québec, Canada)

Introduction

Dexamethasone a synthetic glucocorticoid is used for several applications in different domains such as anti-inflammatory, oncology, obstetrics, and many more. Depending of the application, very sensitive methods may be required in order to assess pharmacokinetic parameters. For the current application, it was necessary to develop a femtogram level detection method for the analysis of dexamethasone in human plasma. Very few methods are published having LOQs under 100 pg/mL.



Dexamethasone

MW: 392.5 g/mole

Method

An extraction method was developed and validated with an analytical range of 0.5 to 1000 pg/mL. 1 mL of human plasma was mixed with internal standard and buffer solution then extracted using 7 mL of MTBE. Labeled dexamethasone was used as internal standard. The sample was concentrated by 10-fold during extraction. Selectivity issues were observed during R&D which were solved by optimizing solvent evaporation conditions. Solvent evaporation had to be optimized to avoid potential cross-contamination in the evaporator due to the wide spreaded analytical range, which directly affected selectivity at such low detection levels. Optimizing these conditions was a critical step in order to reach required sensitivity and selectivity.

Extraction Procedure

Matrix	EDTA K_2
Analytical Range	0.500-1000 pg/mL
Internal Standard	Dexamethasone-d_4
Sample Volume	1.000 mL
Extraction Type	Liquid-Liquid Extraction
Concentration Factor	10

LC-MS/MS Analysis

	Human Method
Chromatographic Mode	Reverse Phase
Analytical Column	ACE Excel 2 C18
Elution Mode	Isocratic
Mobile Phase A	Methanol/Water/Ammonium formate/Formic Acid 0.1%
Flow Rate	0.550 mL/min
Injection Volume	40 uL
Retention Time	1.78 min for Dexamethasone
	1.75 for Dexamethasone-d_4
Acquisition Time	4.00 min
Detector	API 5000
Source	TurboIonSpray
Ion Monitored	393→373 for Dexamethasone
	397→377 for Dexamethasone-d_4

Results

Between-run accuracy and precision was evaluated with mean biases ranging from -3.97% to 1.27% and mean %CV ranging between 3.09% and 14.13% respectively (**Table 1**). Within-run accuracy and precision was also assessed. Mean recovery was of 80% over the analytical range (**Table 2**). Sensitivity was very good with a signal-to-noise ratio of 10/1 at 500 fg/mL (**Figure 1**). Several other validation parameters were evaluated including matrix effects, potentially interfering commonly used drugs, dilution integrity, selectivity and carryover. All tests met acceptance criteria. Stabilities including bench-top, freeze-thaw, long-term and whole blood stabilities were also assessed. The assay was used for study sample analysis with an ISR confirmation rate of 100%.

Table 1. Between-Run Accuracy and Precision

	LLQC 0.50 pg/mL		QC1 1.50 pg/mL		QC2 500.00 pg/mL		QC3 750.00 pg/mL	
	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias
N	60	60	60	60	60	60	60	60
Mean	0.506	1.27	1.507	0.46	480.332	-3.93	726.939	-3.07
SD(±)	0.0715		0.1965		14.8205		24.1078	
CV(%)	14.13		13.04		3.09		3.32	

Table 2. Within-Run Accuracy and Precision

	LLQC 0.50 pg/mL		QC1 1.50 pg/mL		QC2 500.00 pg/mL		QC3 750.00 pg/mL		ULQC 1000.00 pg/mL	
	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias	Measured Conc. (pg/mL)	% Bias
	0.51	2.00	1.51	0.67	460.94	-7.81	723.68	-3.51	967.55	-3.25
	0.45	-10.00	1.48	-1.33	465.24	-6.95	718.78	-4.16	955.35	-4.47
	0.49	-2.00	1.49	-0.67	471.03	-5.79	718.47	-4.20	961.15	-3.89
	0.53	6.00	1.57	4.67	473.23	-5.35	725.36	-3.29	963.84	-3.62
	0.53	6.00	1.47	-2.00	475.96	-4.81	731.95	-2.41	974.84	-2.52
	0.46	-8.00	1.51	0.67	470.76	-5.85	733.57	-2.19	965.56	-3.44
N	6	6	6	6	6	6	6	6	6	6
Mean	0.495	-1.00	1.505	0.33	469.527	-6.09	725.302	-3.29	964.715	-3.53
SD(±)	0.0345		0.0356		5.4953		6.3938		6.5184	
CV(%)	6.97		2.37		1.17		0.88		0.68	

Chromatography

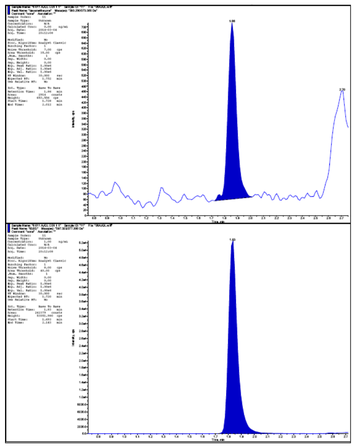


Figure 1. Representative Chromatogram of a Calibration Standard at 0.500 pg/mL in Human EDTA K_2 Plasma

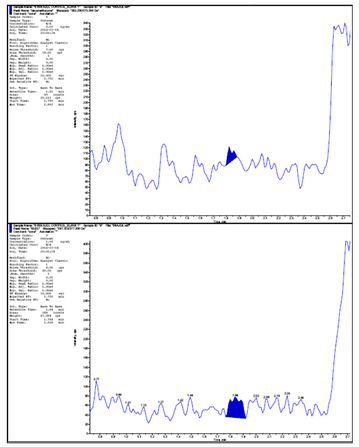


Figure 2. Representative Chromatogram of a Blank Human EDTA K_2 Plasma

Chromatography

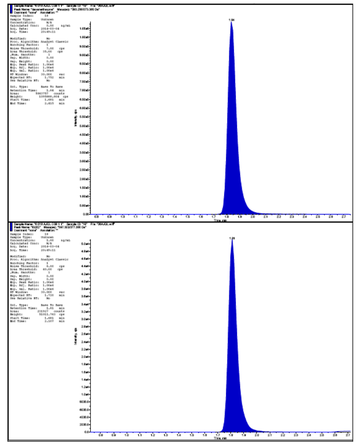


Figure 3. Representative Chromatogram of a Calibration Standard at 1000 pg/mL in Human EDTA K_2 Plasma

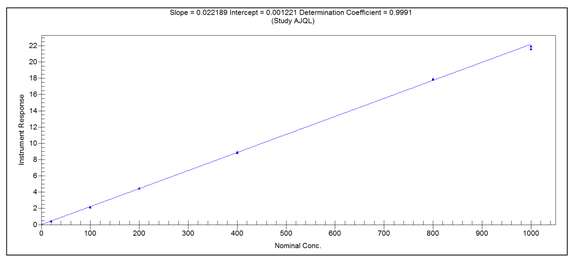


Figure 4. Representative Calibration Curve from 0.500-1000 pg/mL

Conclusion

The assay for dexamethasone in human EDTA K2 plasma was validated as per the most recent validation guidelines over the dynamic range of 0.500 to 1000 pg/mL. The assay was shown to be accurate, precise and highly sensitive. Moreover, the assay was found to be reproducible as the incurred sample reproducibility results showed a reassay confirmation rate of 100%.

TRANSFORMING PROMISING IDEAS INTO COMMERCIAL REALITY