Highly Sensitive LC-MS/MS Method for the Quantification of Fluticasone Propionate in Human plasma, using the SCIEX QTRAP® 6500 System

Sensitive Bioanalytical Quantitation on the SCIEX QTRAP® 6500 LC-MS/MS System

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Key Challenges of Fluticasone Propionate Quantitation

- **Sensitivity**: Due to low circulating levels, it is necessary to have a sensitive method (sub pg level LOQ) to correctly define pharmacokinetic parameters.
- **Sample cleanup:** Removing plasma proteins and phospholipids from the sample to improve assay ruggedness and reproducibility is necessary.
- **Assay Feasibility:** Using high plasma aliquot and lower reconstitution volume can impact re-injection/repeat analysis.
- **Regulatory compliance:** Using relatively large plasma aliquot volumes for assays may require large volume of sample collection from volunteers, which is a great concern for regulatory compliance of clinical studies.

Unique Features of the Method and SCIEX QTRAP® 6500 System

- A highly sensitive and reproducible method was developed for use in regulated bioanalytical labs using a simple solid phase extraction technique.
- The LLOQ for fluticasone propionate in plasma was 0.200 pg/ml with an aliquot volume of 500 µL of plasma.
- Patented IonDrive™ System Technology improves sensitivity and robustness: The patented IonDrive™ technology yields improved ion production, transmission and detection for ultimate sensitivity and reproducibility.
- Next-generation eQ™ Electronics on the SCIEX QTRAP® 6500 provide improved performance at ultra-low MRM dwell times for improved support of fast LC and narrow peak widths.

Figure 1. The SCIEX QTRAP® 6500 System.

INTRODUCTION

Fluticasone propionate is a synthetic glucocorticoid with potent anti-inflammatory activity effectively used in the treatment of chronic asthma and obstructive pulmonary diseases. It is mainly administered via inhalation because of its negligible (<1%) oral systemic bioavailability. Plasma concentrations of fluticasone propionate at therapeutic inhaled dose ranges are extremely low and require sensitive assays to determine the pharmacokinetic parameters.

Systemic exposure data of fluticasone propionate, 88 mcg twice daily dose after inhalation in adults and adolescents (>12 years) shows an average C_{max} of 20 pg/mL which necessitates the use of a sensitive analytical method that can quantify at sub-pico gram per mL levels.

In recent years, many analytical methods have been developed for pharmacokinetic studies or clinical trials of fluticasone propionate, however, to achieve the necessary sensitivity, most of the methods use a large plasma sample aliquot and a low reconstitution volume which limits the feasibility of performing reinjection reproducibility

or repeat analysis in a GLP regulated bioanalytical laboratory.

The main objective of this work is to develop a sub-pico gram level LOQ (200 fg/mL) LC-MS/MS method, feasible for a regulated bioanalytical laboratory, for the quantification of fluticasone propionate in human plasma using fluticasone propionate –D_3 as internal standard.

MATERIALS AND METHODS

Sample Preparation:

Fluticasone propionate standards were prepared in Human K_2EDTA plasma from 0.200 to 120 pg/ml and QC samples were prepared at 0.200, 0.600, 60.0 and 100 pg/mL. Samples (500 µL) were spiked with 50 µL of fluticasone propionate-D_3 (25 pg/mL) as internal standard solution and were subjected to protein precipitation followed by reverse phase SPE purification using Cleanert S C18-SPE cartridge. After loading, the samples were washed with water followed by 25% methanol twice and then eluted using dichloromethane. The eluent was dried under a stream of nitrogen at 40ºC. Dried samples were then reconstituted with 200 µL of mobile phase for LC/MS analysis.

Table 1. Chromatographic conditions

Column	Phenomenex Kinetex C18 (100mm × 3mm, 2.6 µm)	
Mobile Phase	Acetonitrile / Ammonium Trifluoroacetate buffer	
Flow rate	600 µL/min	
Column temperature	45 °C	
Injection volume	50 µL	
LC system	Shimadzu Nexera 30AD	
Gradient profile	**Time (min)**	**% B**
	0.01	50
	1.50	50
	3.00	60
	3.50	70
	4.00	95
	5.00	50
	7.00	50

Mass spectrometric conditions:

The SCIEX QTRAP® 6500 mass spectrometer was operated in positive electrospray ionization mode. The MS conditions were as follows: scan type positive MRM, Q1 resolution at unit and Q3 at unit; curtain gas set at 25; ion source temperature 400°C, ion source gas (GS1) at 75 and drying gas (GS2) at 70; ion spray voltage at 3000 V; and dwell time 200 ms for all transitions. The compound dependent parameters for analyte and internal standard were as follows,

Table 2. Mass spectrometry conditions

COMPOUND	Q1	Q3	DP	EP	CE	CXP
Fluticasone Propionate	501.2	293.2	55	10	22	8
Fluticasone Propionate	501.2	313.2	55	10	19	8
Fluticasone Propionate -D3	504.2	313.2	55	10	19	8

Data Processing

Analyst® Software version 1.6 was used for mass spectrometer data acquisition and Multiquant™ Software 3.0.2 for processing. A $1/x^2$ weighted linear regression was used to calculate the concentrations.

RESULTS AND DISCUSSIONS

Fluticasone propionate produced two intense product ions at *m/z* 293.2 and 313.2 and both were selected for summation of peak area response and used for quantitation and the fluticasone propionate-D_3 (IS) product ion at 313.2 was selected. The mass spectrometric parameters for both analyte and internal standard were given in Table 2.

Various sample extraction techniques like liquid-liquid extraction, protein precipitation and SPE were tried to separate the analyte of interest from the matrix components. The SPE technique outlined above produced promising results over the published sample prep methods because it removed a significant number of matrix components as evidenced by the zone free of matrix suppression (Figure 2A & 2B) at the analyte and IS retention time.

In the present method, linearity was established in the range of 0.200 to 120 pg/ml in human plasma. The calibration curve is shown in figure 3 with correlation coefficient r = 0.99. Table 2 shows the accuracy and precision data at different QC levels of fluticasone propionate. All are within the acceptance criteria of %CV ±20% at LLOQ level and ±15% at other levels. Example chromatograms of the blank, LLOQ and ULOQ calibration standards are shown in figure 4.

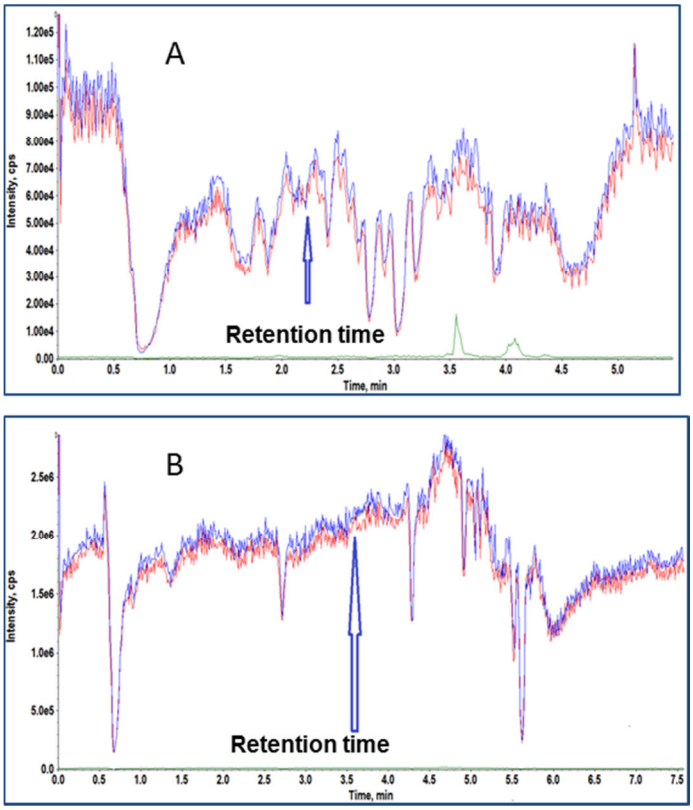

Figure 2. T-Infusion experiment using conventional (A) and our optimized (B) SPE extraction technique that shows reduced matrix suppression at the retention time of fluticasone propionate.

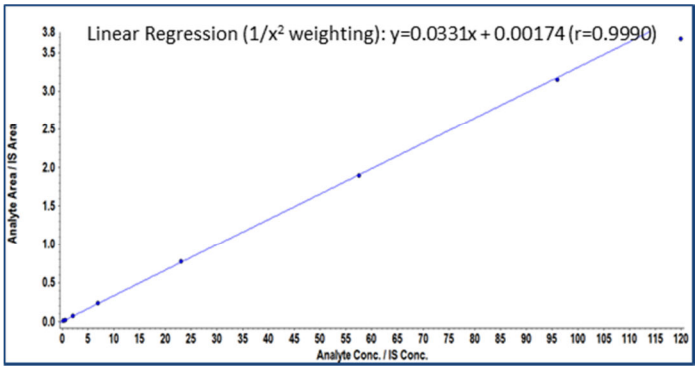

Figure 3. Calibration curve of fluticasone propionate from 0.200 to 120 pg/mL.

Table 2. Mass spectrometry conditions

Sample Number	LOQ QC (pg/ml)	LQC (pg/ml)	MQC (pg/ml)	HQC (pg/ml)
1	0.19	0.57	58.44	92.60
2	0.17	0.59	61.04	95.75
3	0.24	0.60	55.99	93.52
4	0.20	0.60	57.91	89.89
5	0.27	0.56	54.46	89.70
6	0.25	0.60	55.26	89.37
7	0.25	0.58	56.39	90.76
8	0.20	0.60	58.49	94.33
9	0.18	0.62	53.54	88.25
10	0.20	0.56	55.33	90.03
11	0.24	0.58	54.84	87.24
12	0.22	0.62	53.90	89.41
Mean	**0.218**	**0.590**	**56.299**	**90.904**
S.D (+/-)	**0.0319**	**0.0204**	**2.2453**	**2.5806**
C.V (%)	**14.69**	**3.47**	**3.99**	**2.84**
Nominal	**0.20**	**0.58**	**57.60**	**96.00**
Accuracy (%)	**108.75**	**101.72**	**97.74**	**94.69**

CONCLUSIONS

A highly selective, sensitive and reproducible bioanalytical method was developed for the detection of fluticasone propionate with an LLOQ of 200 fg/mL in human plasma with the SCIEX QTRAP® 6500 LC-MS/MS System. A key property of this method is the required sample volume (500 µL plasma), which is lower than most other methods plus the final reconstitution volume of 200 µL makes this method amenable for reinjection of samples or repeat analysis chromatography if required in a GLP laboratory.

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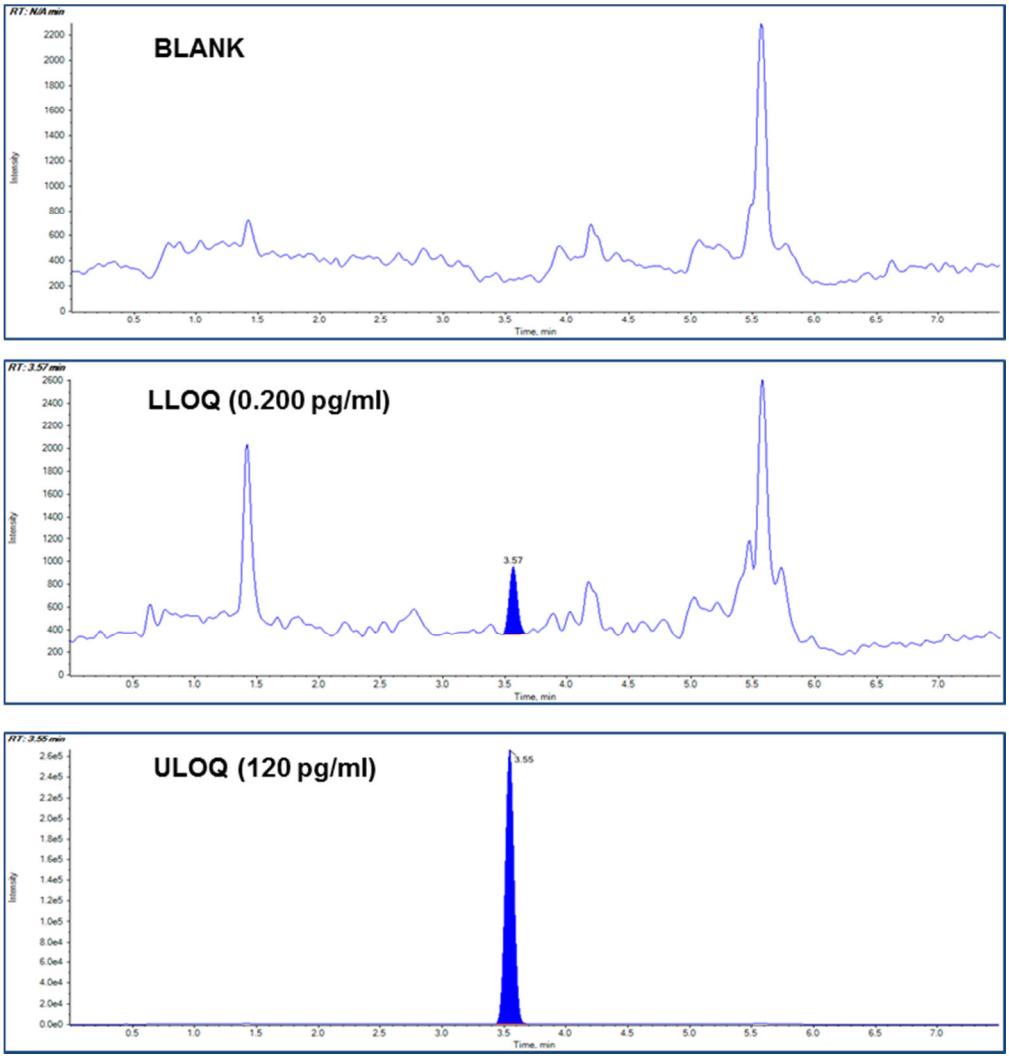

Figure 6. Chromatograms of blank plasma, LLOQ and ULOQ samples from the fluticasone propionate method.

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