Determination of Fluticasone Propionate and Fluticasone 17&[beta]-Carboxylic Acid Propionate (Fluticasone Metabolite) in Human Plasma by LC/MS/MS

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PURPOSE

Fluticasone propionate belongs to corticosteroids class of drugs. It is most effective treatment for persistent asthma. Major metabolite for Fluticasone propionate is Fluticasone 17β-Carboxylic Acid Propionate and is excreted in urine.
In this study we developed and validated a LC-MS/MS method for the determination of Fluticasone propionate and Fluticasone 17β-carboxylic acid propionate (Fluticasone metabolite) in human K3EDTA plasma using Fluticasone propionate-d5 and Fluticasone 17β-carboxylic acid propionate-d3 as the internal standards (IS) using positive and negative TIS switch during analysis.

Chemical structures

Figure A: Fluticasone Propionate
Figure B: Fluticasone 17β-Carboxylic Acid Propionate
Figure C:Fluticasone propionate-d5
Figure D:Fluticasone 17β-Carboxylic acid propionate-d3

Figure A

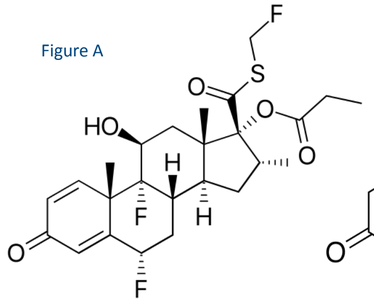


Figure B

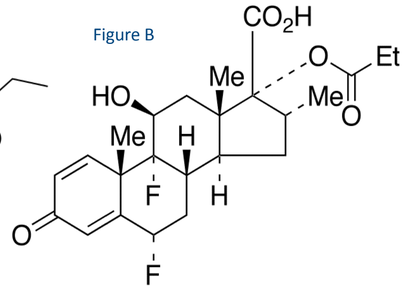


Figure C

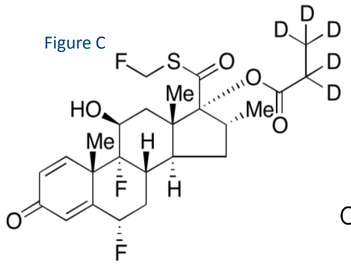
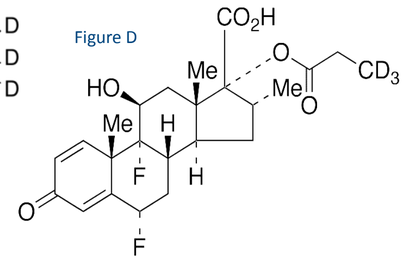


Figure D



METHOD

Calibration range for Fluticasone Propionate is 5-5,000 pg/mL and 20-20,000 pg/mL for Fluticasone 17ß-carboxylic acid propionate. Analytes and the IS were extracted by solid phase extraction from human K3EDTA plasma using CEREX Trace-N cartridges. MS/MS detection was set first for Fluticasone 17ß-carboxylic acid propionate and Fluticasone 17ß-carboxylic acid propionate-d3 in TIS negative mode. Subsequently the ionization mode was switched to TIS positive mode with MS/MS detection set for Fluticasone propionate and Fluticasone propionate-d5.

Instruments:

Shimadzu HPLC prominence with Autosampler
AB Sciex® API5000 Mass spectrometer

Extraction details:

Extraction type:	Solid Phase Extraction
Sample volume:	400 µL
Buffer:	0.001N Hydrochloric acid
Cartridges:	CEREX Trace-N SPE cartridges
Elution Solvent:	1% Ammonium Hydroxide solution

Chromatography Details:

Mobile Phase A:	0.3% Ammonium Hydroxide in purified H_2O
Mobile Phase B:	Pure Acetonitrile
Flow rate:	0.5 mL/min
Injection Volume:	20 µL
HPLC column:	Waters, Xbridge C-18, 2.1 x 50 mm, 3.5µ

HPLC Gradient Details:

Time	Flow rate:	%B
0 min	0.5 mL/min	20
2.0 min	0.5 mL/min	100
3.0 min	0.5 mL/min	100
3.1 min	0.5 mL/min	20
4.8 min	0.5 mL/min	20

MS/MS detection:

MS/MS detection was achieved using Multiple Reaction Monitoring (MRM) scans in TurboIonSpray (TIS) positive and negative mode.

Positive TIS

Analyte	Q1 → Q3
Fluticasone Propionate:	501.3→313.2
Fluticasone Propionate -d5:	506.3→313.2

Negative TIS

Analyte	Q1 → Q3
Fluticasone 17β-Carboxylic Acid Propionate:	451.1→395.2
Fluticasone 17β-Carboxylic Acid Propionate-d3:	454.1→395.2

RESULTS

Fluticasone bio-assay was developed and validated at Frontage Laboratories according to FDA guideline for Validation of Bioanalytical Method.

Linearity:

R2 ≥ 0.9890 for Fluticasone
R2 ≥ 0.9977 for Fluticasone 17ß-carboxylic acid Propionate

Selectivity:

No interfering peaks were detected at analyte retention times.

Matrix Effect (IS-normalized Matrix factor):
Fluticasone Propionate
0.90 ± 0.02 at 15.0 pg/mL with %CV = 2.2%
1.23 ± 0.06 at 3,750 pg/mL with %CV = 4.9%
Fluticasone 17ß-carboxylic acid Propionate
1.02 ± 0.02 at 60.0 pg/mL with %CV = 2.0%
1.05 ± 0.01 at 15,000 pg/mL with %CV = 1.0%

Inter-conversion:

There was no significant inter-conversion from Fluticasone17ß- Carboxylic acid Propionate to Fluticasone Propionate, or vice versa.

Figure E, F: Representation of Precision and Accuracy results:

Figure E

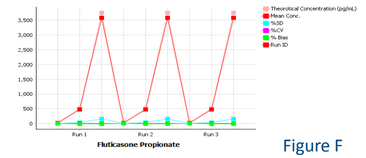


Figure F

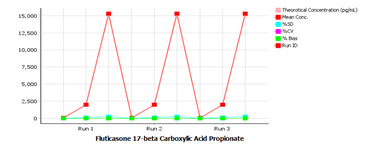


Table 1:Precision and Accuracy Results

Fluticasone Propionate

	15 pg/mL	600 pg/mL	3750 pg/mL
Inter-run Mean	14.9	512	3560
Inter Run SD	1.71	40.1	171
Inter-run %CV	11.5	7.8	4.8
Inter-run % Bias	-0.7	2.4	-5.1
n	18	18	18

Fluticasone 17β-Carboxylic Acid Propionate

	60 pg/mL	2000 pg/mL	15000 pg/mL
Inter-run Mean	62.1	2050	15300
Inter Run SD	5.66	125	453
Inter-run %CV	9.1	6.1	3.0
Inter-run % Bias	3.5	2.5	2.0
n	18	18	18

Typical Chromatograms for LLOQ samples:

Figure G

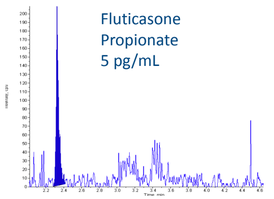


Figure H

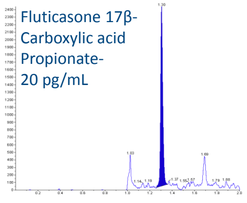


Table 2: Stability Results

	Fluticasone Propionate	Fluticasone 17β-Carboxylic Acid Propionate
QC Bench Top Stability	6 hours	6 hours
Processed Sample Stability	54 hours at 4°C	54 hours at 4°C
Reinjection Stability	115 hours at 4°C	115 hours at 4°C
Freeze Thaw Stability	3 freeze thaw cycles for -20°C and -70°C	3 freeze thaw cycles for -20°C and -70°C
Long Term Storage Stability	216 days at -20°C 245 days at -70°C	216 days at -20°C 245 days at -70°C

CONCLUSION

A sensitive bioanalytical assay was developed and validated for determination of Fluticasone and Fluticasone 17β-Carboxylic Acid Propionate in human plasma by LC-MS/MS. A simultaneous TIS switch from negative to positive was used to detect analyte signal during the injection. The validated method is robust and have been successfully applied to multiple GLP studies. Successful incurred sample reproducibility (ISR) demonstrated that method is reliable for analyzing Fluticasone propionate and Fluticasone 17β- Carboxylic acid propionate.

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