Fluticasone propionate belongs to corticosteroids class of drugs. It is most effective treatment for persistent asthma. Major metabolite for Fluticasone propionate is Fluticasone 17ß-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 17ß-carboxylic acid propionate (Fluticasone metabolite) in human K3EDTA plasma using CEREX Trace-N SPE cartridges. MS/MS detection was set first for Fluticasone 17ß-carboxylic acid propionate and Fluticasone 17ß-carboxylic acid propionate-d5 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.

**Chemical structures**

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

 purs: Shimadzu HPLC prominence with Autosampler  
All Sort® API5000 Mass spectrometer

**Extraction details:**

Extraction type: Solid Phase Extraction  
Sample volume: 400 µL  
Buffer: 0.003N 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 H₂O  
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  
0 min 0.5 mL/min  
2.0 min 0.5 mL/min  
3.0 min 0.5 mL/min  
3.1 min 0.5 mL/min  
4.8 min 0.5 mL/min

**MS/MS detection:**

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

**METHOD**

Calibration range for Fluticasone Propionate is 5,000 pg/mL and 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-d5 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.

**RESULTS**

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

**Linearity:**

<table>
<thead>
<tr>
<th>R²</th>
<th>R²</th>
<th>R²</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.90 ± 0.02 ± 0.05 ± 0.06 ± 0.15</td>
<td>0.99 ± 0.17 ± 0.22 ± 0.23 ± 0.32</td>
<td>0.98 ± 0.13 ± 0.24 ± 0.25 ± 0.34</td>
<td>0.99 ± 0.17 ± 0.22 ± 0.23 ± 0.32</td>
</tr>
</tbody>
</table>

**Selectivity:**

No interfering peaks were detected at analyte retention times.

**Stability:**

Typical Chromatograms for LLOQ samples:

**CONCLUSION**

A sensitive bioanalytical assay was developed and validated for determination of Fluticasone and Fluticasone 17ß-Carboxylic Acid Propionate in human plasma by LC/MS/MS. A simultaneous TS 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 17ß-Carboxylic acid propionate.