Fluticasone propionate (FP) is an inhaled bronchodilator used to reduce the inflammation that leads to symptoms including congestion, sneezing, itchy, and runny nose. Ten years ago, we developed an LC-MS/MS method capable quantifying 3 pg/mL of FP in human plasma. The method has been successfully applied to pharmacokinetic studies involving human plasma samples.

**Overview**

A sensitive and specific liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method capable of quantifying fluticasone propionate in human plasma is described.

In this method, 0.6 mL of plasma was used. The drug was extracted from plasma using a reverse phase C18 solid phase extraction method. The separation was performed on a reverse phase C8 column. Detection was achieved using an AB SCIEX API-5000 tandem mass spectrometer, employing positive ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lowest limit of quantitation was 1.00 pg/mL. The method was fully validated per FDA guidelines in the lower limit quantification of 1.00 pg/mL with only 0.6 mL of plasma sample. The method transfer from API-3000 to API-5000, was applied for monitoring the deprotonated molecular ion. With an API-3000 system, a low background and good signal to noise ratio was obtained. A possible reason is that the positive mode was, therefore, employed in which the protonated molecular ion ([M+H]+) appeared to have little effect on the quantitation. In our previous work, electron spray negative mode was applied for monitoring the deprotonated molecular ion. With an AP-3000 system, a low background and good signal to noise ratio were obtained in negative mode. To reach a lower quantitation limit, an AP-5000 was utilized. The simple method transfer from AP-3000 to AP-5000, however, could not reach this quantitation level. In the negative mode, we have not only observed the ion [M-H]−, but also [M+Cl]− and etc. as shown in Figure 2A. In our previous work, electron spray negative mode was applied for monitoring the deprotonated molecular ion. With an AP-3000 system, a low background and good signal to noise ratio were obtained in negative mode. To reach a lower quantitation limit, an AP-5000 was utilized. The simple method transfer from AP-3000 to AP-5000, however, could not reach this quantitation level. In the negative mode, we have not only observed the ion [M-H]−, but also [M+Cl]− and etc. as shown in Figure 2A.

**Introduction**

Fluticasone propionate (FP) is an inhaled bronchodilator used to reduce the inflammation that leads to symptoms including congestion, sneezing, itchy, and runny nose. Ten years ago, we developed an LC-MS/MS method capable quantifying 3 pg/mL of FP in human plasma. The method has been successfully applied to pharmacokinetic studies involving human plasma samples.

**Sample Preparation**

Fluticasone propionate and the internal standard (fluticasone propionate-D3) were mixed with the buffer solution and transferred onto the preconditioned Strata C18-E Polymer 96-well plate (Phenomenex). The plate was preconditioned with 0.8 mL of methanol and 0.8 mL of water. The loaded plates were washed with 1.6 mL of water, and 0.8 mL of 20% methanol in water. Both analyte and internal standard were then eluted using 900 µL methanol in water.

**LC-MS/MS Conditions**

- **Liquid Chromatography:** Shimadzu LC-20A
- **Analytical Column:** Hypersil 3 µm, 50 x 2.00 mm, 3 µm
- **Mobile Phase A:** Water with 0.04% ammonium hydroxide
- **Mobile Phase B:** Acetonitrile
- **Flow rate:** 0.7 mL/min
- **Injection Volume:** 10 µL
- **Gradient:**
  - Linear: 0% B to 100% B in 5 min
  - Linear: 100% B to 0% B in 5 min
  - Linear: 0% B to 100% B in 5 min
- **Method Recovery**
  - Inter-Batch (n=18)
  - Accuracy & Precision
  - Medium 80.0 -3.75 3.47
  - High 160 -6.25 4.00
  - Low 3.00 -1.67 8.68
- **Limit of quantitation:** 1.00 pg/mL

**Results and Discussion**

The eluted samples were evaporated for approximately 30 min in a 96-Well Nitrogen evaporator (Eppendorf®) at about 35 °C. The residue was redissolved in 125 µL of 50% methanol in water.

A sensitive and specific LC-MS/MS method was developed and validated for measuring fluticasone propionate in plasma. Both analyte and internal standard were then eluted using 900 µL methanol in water.

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**Structures**

Figure 3. Ion chromatograms of blank plasma (A) and LLOQ (1 ng/mL) plasma sample (B) with good clean up.