A RAPID METHOD FOR THE ULTRA-SENSITIVE QUANTIFICATION OF FLUTICASONE PROPIONATE AND SALMETEROL XINAFOATE FROM HUMAN PLASMA

INTRODUCTION
Fluticasone propionate (Figure 1a) (1) is a synthetic inhaled glucocorticoid receptor agonist with anti-inflammatory and anti-secretory effects (2). Fluticasone propionate binds to and activates the glucocorticoid receptor resulting in the activation of specific transcription factors that inhibit cytokine and inflammatory mediator synthesis; such as prostaglandins and leukotrienes. Salmeterol xinafoate (Figure 1b) (3) is a highly selective, long-acting beta2-agonist with bronchodilatory activity (4). It is used in the management and prevention of asthma symptoms and maintenance of chronic obstructive pulmonary disease (COPD) symptoms. These two inhaled compounds are often co-administered in the treatment of asthma and COPD. Both compounds were approved by the U.S. Food and Drug Administration (FDA) for use in systemic exposure (5). Most of the systemic exposure is due to the patient inhaling the dose and the molecule entering the bloodstream via the portal vein and liver. These levels are extremely low with peak concentrations of sub pg/mL reported, thus requiring a very high sensitivity assay to detect these compounds. Due to the extremely low circulating levels of these drugs, there is always a need to develop high sensitivity assays to better understand treatment concentrations. Previously published method for these molecules have achieved LLOQ’s of 0.2 pg/mL (6) and 0.1 pg/mL for Fluticasone propionate and Salmeterol xinafoate respectively. This poster describes an optimized, quick and simple workflow using Oasis PRiME µElution, Acquity UPLC BEH C18, achieving LLOQ of 0.1 pg/mL and 0.05 pg/mL for Fluticasone propionate and Salmeterol xinafoate respectively.

METHODS

**SAMPLE PREPARATION:**

Concentrated Plasma Samples were added onto the extraction plate in two steps of ~ 400 µL each. Wash: 200 µL of 50% Methanol in Water. Dilute: 2.25 µL of 1000ppb Methanol in Water.

**LC CONDITIONS:**

- **Detector:** Acquity UPLC I-Class
- **Column:** Acquity UPLC BEH C18, 130A, 1.7 µm, 2.1 mm x 50 mm
- **Sample Temp:** 5 °C
- **Mobile-Phase:** A: 10% Ammonium Hydroxide in Water B: 10:90 Isopropanol/Methanol (%)

**MS CONDITIONS:**

- **Flow Rate:** 500 µL/min
- **Sample Injection Volume:** 6 µL
- **Peak Width:** 6 sec
- **Peak Area:** 1.13 e6
- **Flow Rate:** 500 µL/min
- **Peak Width:** 5 sec
- **Peak Area:** 17.61 e5

**RESULTS**

Using 400 µL of plasma and the aforementioned sample preparation strategy, quantification limits of 0.1 pg/mL & 0.05 pg/mL (Figures 2a & 2b) for Fluticasone propionate and Salmeterol xinafoate respectively were achieved. Calibration curves were linear with R² values > 0.99 (1 weighted regression) with inter-day mean accuracies of 100% and 99.32% for Fluticasone propionate and Salmeterol xinafoate respectively. A summary of standard curve performance is shown in Table 1a & b. In addition, intra and inter-day precision and accuracy for both analytes was excellent with mean RSD’s of ±10%. QC performance is highlighted in Table 2a (Figure 2b) and 2b (Salmeterol xinafoate) and a representative QC chromatogram for Fluticasone propionate is shown in Figure 3.

**DISCUSSION**

Sample Preparation: Fluticasone propionate is known to have extremely high protein binding (>95%) (7). Hence, it is essential to dissociate the analyte from plasma proteins to ensure accurate quantification of circulating levels. This was achieved by diluting the plasma sample with a combination of Ammonium Hydroxide and Zinc Sulphate. SPE protocol previously developed by Molter et al. (6) was used as the starting point for method development. Various wash solvent compositions, starting from 10:90 to 50:50 Methanol/Water (v/v) were evaluated. The use of Oasis PRiME µElution as the wash solvent yielded the best area counts and was employed for the final method. Similarly, different elution solvents, comprising of 10:50 to 75% Isopropanol Methanol, 25% to 75% Acetone/Methanol, 100% acetone and 100% Isopropanol were evaluated. 150 µL and 20:50 Isopropanol/Methanol (v/v) were the highest recovery and area counts yielding the best equilibrium steps, making the sample preparation process simpler and quicker. For Fluticasone propionate and Salmeterol xinafoate, use of Oasis PRiME HLB showed matrix effects <1% and recoveries of 90% and was used in the final protocol.

**Liquid Chromatography-Mass Spectrometry**

The physico-chemical properties of Fluticasone propionate and Salmeterol xinafoate make them ideally suited for a reversed-phase chromatographic separation. Multiple reversed-phase columns, including BEH C18, HSS T3, HSS C18 and CORTECS C18 were evaluated and BEH C18 gave the best chromatographic performance for both analytes. Additionally, flow rate and gradient conditions can also have a significant impact on peak shapes and signal to noise. After evaluating flow rates from 100 - 500 µL/min and different gradient starting conditions, flow rate of 200 µL/min and initial gradient conditions of 50:50 mobile phase A:B were employed (Figure 4). The Xevo TQ-TOF Ultra HE Q-TOF tandem mass spectrometer operating in positive ion electrospray mode was used to quantify Fluticasone propionate and Salmeterol xinafoate. Source conditions and tune page parameters were optimized and MS/MS transitions listed in the methods section were used.

**CONCLUSION**

The method described employs a simple pretreatment and SPE sample preparation strategy combined with analytical LC and tandem-MS detection, delivering ultra sensitive quantitation of Fluticasone propionate and Salmeterol xinafoate from human plasma. The main features of the method include:

- Simple, fast and cheap sample preparation with simple Oasis PRiME HLB, 4 µL of 50% Ammonium Hydroxide, 500 µL/min and 150 µL equilibration steps.
- Ultra sensitive quantitation of Fluticasone propionate and Salmeterol xinafoate from human plasma.
- LC/MS/MS methods described reliably meet the biosimilarity definition of biosimilars.

**REFERENCES**

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