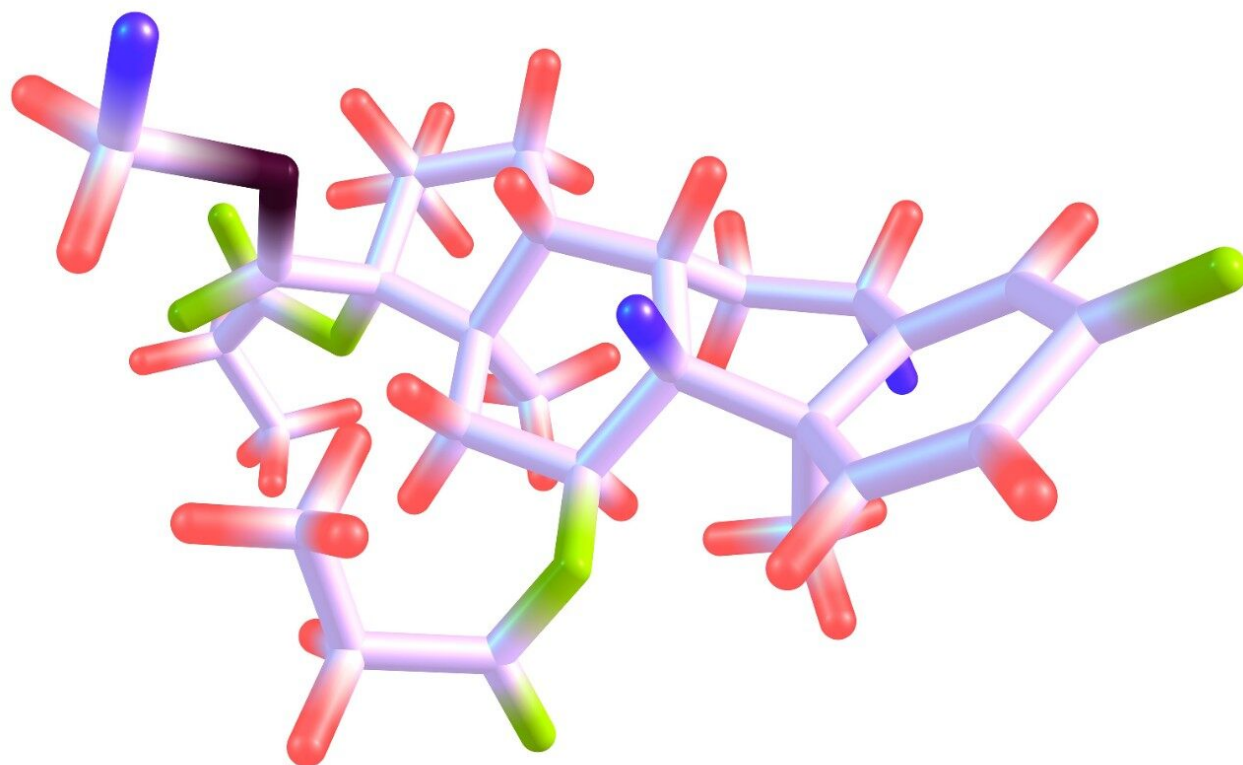


Application Note

Sub-pg/mL Bioanalytical Quantification of Fluticasone Propionate Using the ACQUITY UPLC I-Class System and Xevo TQ-XS Tandem Quadrupole Mass Spectrometer with UniSpray Ionization

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates that the Xevo TQ-XS quadrupole Mass Spectrometer used for quantification of fluticasone propionate (extracted from human plasma) achieved excellent sensitivity and dynamic range, demonstrating its suitability for bioanalytical quantification.

Benefits

Demonstrate sensitive and robust quantitative performance of the Xevo TQ-XS mass spectrometer to achieve sub-pg/mL detection for fluticasone extracted from plasma.

Introduction

Fluticasone propionate (Figure 1) is a potent synthetic trifluorinated corticosteroid with anti-inflammatory activity and is used in the treatment of asthma.¹ Due to its mechanism of action and route of administration, via nasal inhalation, circulating plasma concentrations are low, in the pg/mL range, making accurate quantification challenging.² This work presented herein, highlights a simple and selective solid-phase extraction (SPE) sample preparation, UPLC chromatographic separation, and tandem quadrupole mass spectrometer (MS) with Unispray ionization for sensitive and robust quantification of fluticasone propionate from plasma. This method achieves lower limits of detection (LODs) of 0.2 pg/mL with a linear dynamic range from 0.2-20 pg/mL, extracted from 600 µL of plasma.

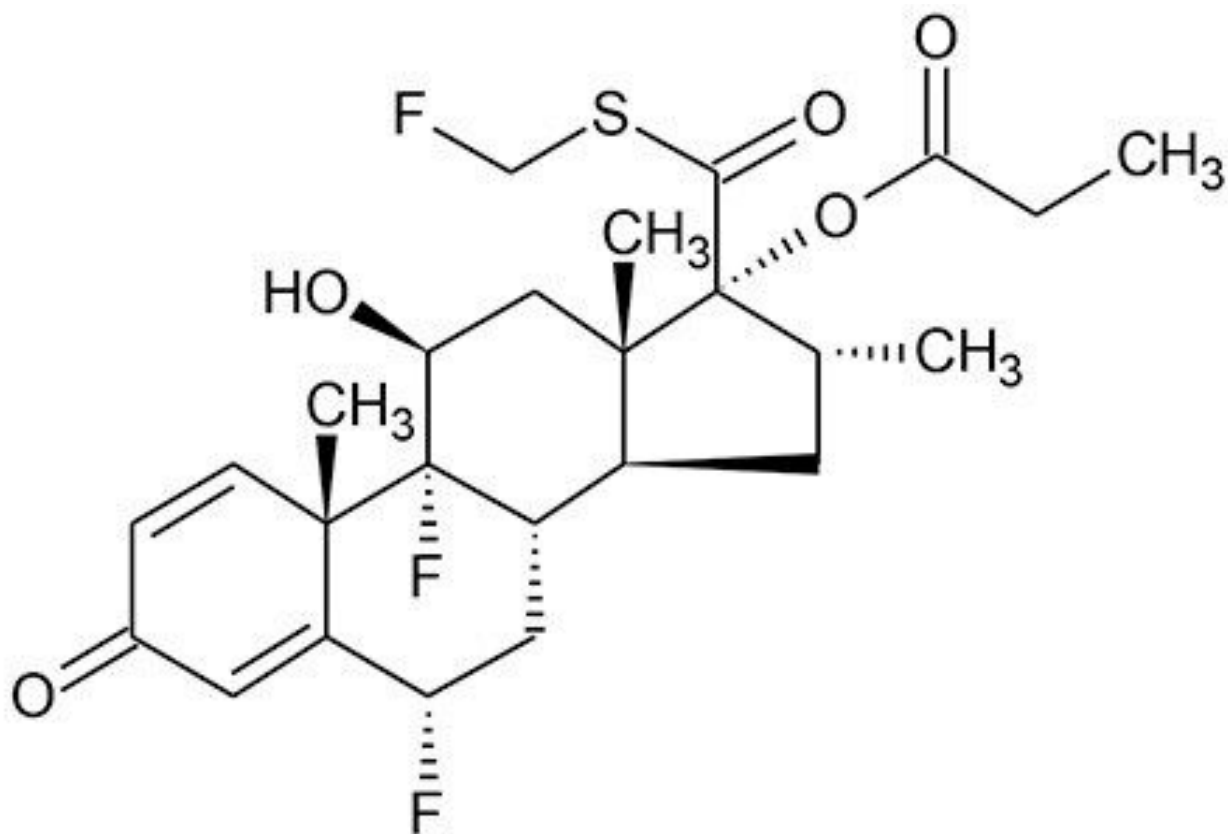


Figure 1: Fluticasone propionate chemical structure.

Results and Discussion

For MS analysis, a multiple reaction monitoring (MRM) experiment was performed on the Xevo TQ-XS. Chromatographic separation was achieved using an ACQUITY UPLC I-Class System and ACQUITY UPLC BEH Phenyl 1.7 μ m Column (p/n 186002885). Fluticasone plasma samples (0.2 to 20 pg/mL) were prepared using commercially available human plasma. Calibration curve standards were prepared in duplicate to check the reproducibility, while six replicates were prepared for the QC and blank (non-spiked) plasma samples. No internal standard was used. A 600 μ L aliquot of each of the prepared plasma samples was pre-treated with 2% formic acid in water and mixed. The pre-treated plasma sample was extracted using the Oasis MCX Cartridges and extraction protocol shown in Figure 2. Following the extraction, 20 μ L of extracted sample was injected for LC-MS/MS analysis.

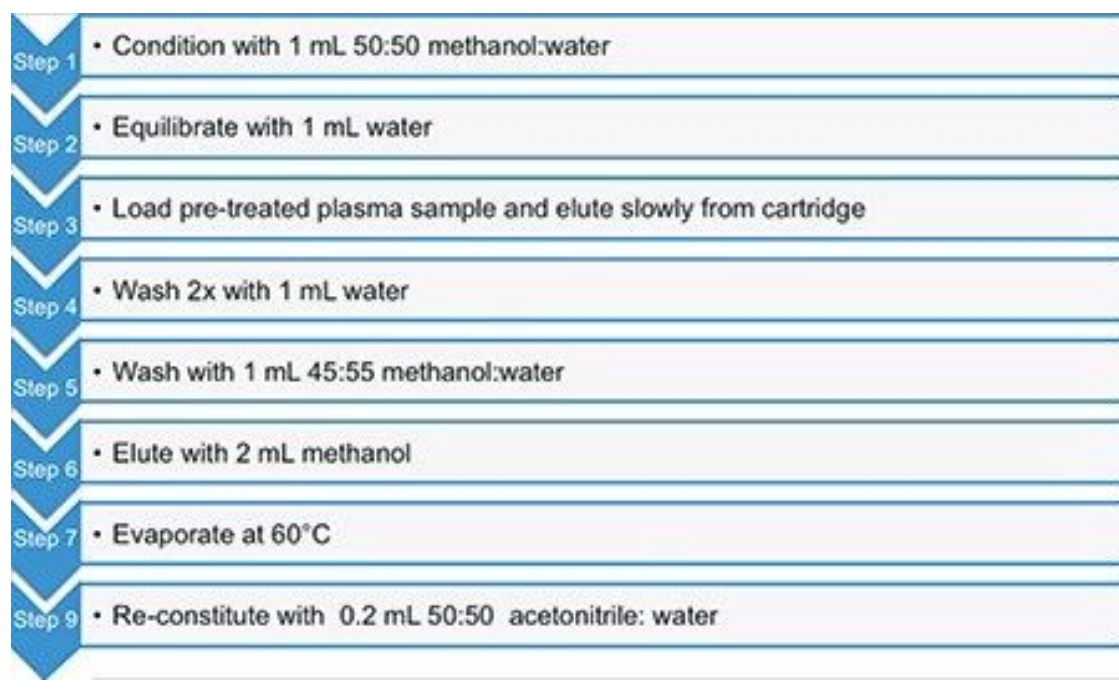


Figure 2. Oasis MCX SPE extraction protocol for fluticasone propionate extracted from human plasma.

Using this SPE UPLC-MS method, quantitative performance was excellent. SPE recovery for fluticasone propionate, using the MCX cartridges and described protocol, was $\geq 85\%$. Chromatographic separation using the low dispersion ACQUITY UPLC I-Class System and BEH Phenyl 1.7 μm Column, provided excellent resolution and separation from endogenous matrix interferences. The Xevo TQ-XS tandem quadrupole mass spectrometer, equipped with a novel StepWave ion guide, enabled improved ion sampling in the source and ion transfer efficiency, while use of the UniSpray ionization technique improved sample ionization improving signal-to-noise (S/N) and detection limits. With this method, lower limits of quantification (LLOQ) of 0.2 pg/mL were achieved with a S/N of 12.

Overall quantification performance was excellent. The method delivered a linear calibration response over the range of 0.2–20 pg/mL (Figure 4) with a correlation coefficient of 0.9960 (Figure 3). At the same time, QC statistics for PA batches easily met recommended bioanalytical method development guidelines 3, with accuracy values $\leq 15\%$ with excellent single digit precision ($\leq 6\%$) for all QC levels (Table 1).

Compound name: FLUTICASONE_2
 Correlation coefficient: $r = 0.998033$, $r^2 = 0.996070$
 Calibration curve: $3548.68 \times x + 493.607$
 Response type: External Std. Area
 Curve type: Linear, Origin: Exclude, Weighting: $1/x^2$, Axis trans: None

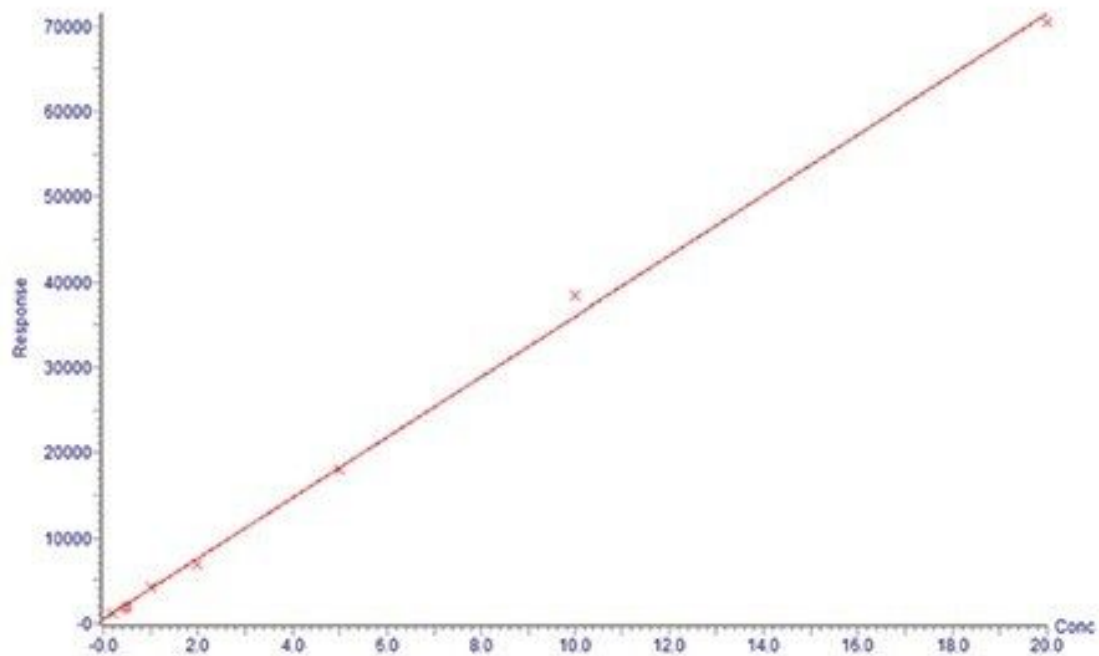


Figure 3. Fluticasone propionate calibration curve from 0.2 to 20 pg/mL extracted from plasma.

| Fluticasone Propionate | | | |
|------------------------|---|-----------------------|-------|
| QC Level | Mean calculated concentration (N=6) pg/mL | % Mean (N=6) accuracy | % RSD |
| LLOQ | 0.2300 | 114.3 | 3.62 |
| LQC | 0.4600 | 91.45 | 6.14 |
| MQC | 1.9500 | 97.7 | 2.62 |
| HQC | 10.8200 | 108.15 | 1.02 |

Table 1. Summary of quality control results for fluticasone propionate extracted from human plasma.

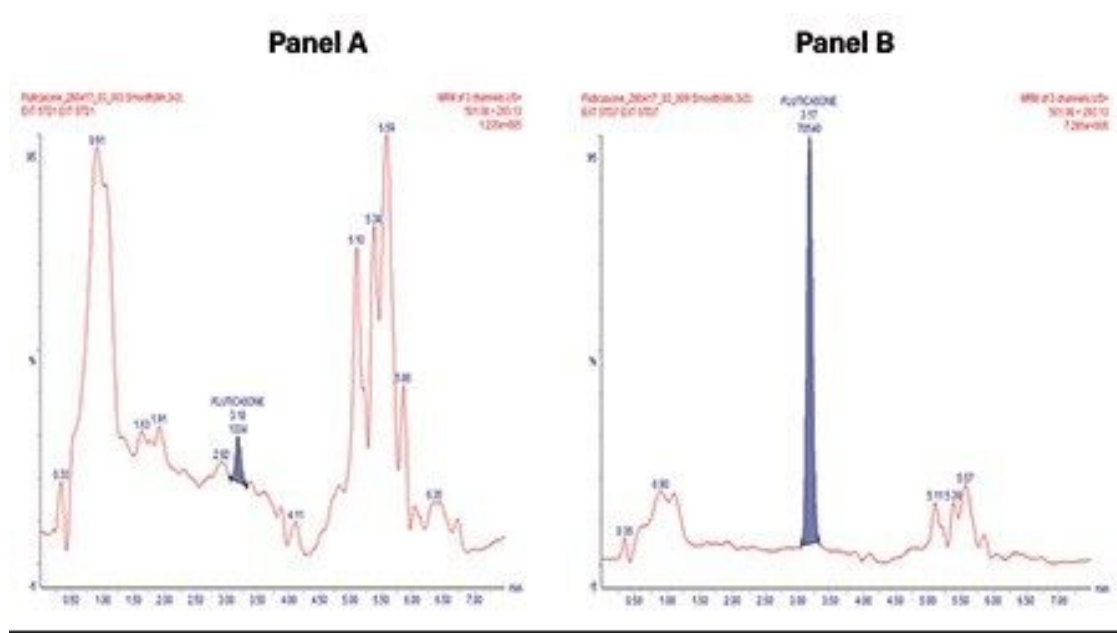


Figure 4. Representative chromatograms for the LLOQ 0.2 pg/mL (Panel A) and ULOQ 20 pg/mL (Panel B) for fluticasone propionate extracted from plasma using the 501.3>293.1 MRM transition.

Conclusion

In this work, a simple sample preparation strategy with Oasis MCX SPE combined with UPLC chromatographic separation ACQUITY UPLC I-Class System, and sensitivity MS analysis, using the Xevo TQ-XS Mass Spectrometer was used to successfully quantify fluticasone propionate from plasma. This method achieved sub-pg/mL detection sensitivity, achieving LLOQs of 0.2pg/mL with a linear dynamic range 0.2-20pg/mL. The excellent quantitative performance of the method described herein, reliably measures low levels of fluticasone propionate, demonstrating its suitability and fit-for-purpose in support of drug discovery and research.

References

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2. J Allanson, et al. A Sensitive LC-MS/MS Method for the Quantitation of Fluticasone Propionate in Human Plasma. Poster Presentation. Land 'O'Lakes Bioanalytical Conference, WI, USA, 2009.

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