# Increased Ion Sampling Efficiency Allows Streamlining Sample Preparation for the Determination of Fluticasone Propionate in Human Plasma

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## Introduction

*Fluticasone Propionate is an inhaled glucocorticoid used in asthma control. Due to its low systemic levels, very sensitive analytical methods are required for its quantitation in human plasma. Typically time-consuming solid phase extraction and liquid-liquid extraction procedures are used in order to concentrate the analyte and to eliminate matrix effects, achieving LLOQs in the order of 5-10 pg/mL. In this work we show how increased MS sensitivity, if not required to lower the limit of detection, can be used to relax the requirements on the sample preparation procedure. Increased ion sampling efficiency was achieved using a new triple quadrupole mass spectrometer in which the standard front end ion optics were replaced with a hexabore capillary and a dual ion funnel. The 6490 also provides enhanced quadrupole resolution for MRM quantitation*

## Experimental

*Sample preparation*

*A calibration curve in the range of 5 to 5000 pg/mL fluticasone propionate in human plasma was prepared. The spiked plasma (100 µL) was prepared by plasma protein precipitation (PPT) as follows: Plasma was precipitated with 3 parts of acetonitrile, centrifuged during 15 min. at 13200 rmp and 200 µL of the supernatant diluted with 4 parts of water.*

*Agilent 1290 Infinity UHPLC:*

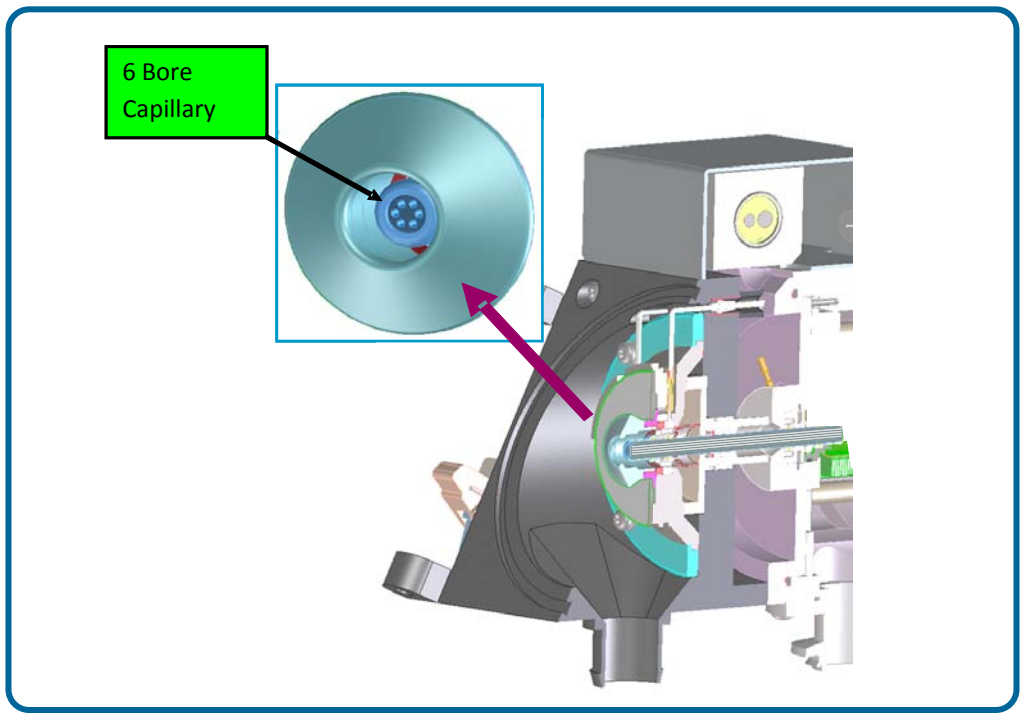
*Column: Agilent Rapid Resolution High Definition (RRHD) Zorbax Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm*
*Mobile phase: A= 0.05% Ammonium Hydroxide in water, B= Methanol; Injection volume: 10 µL; Flow rate: 0.5 mL/min*
*Gradient: 50%B to 100%B in 2 min, 100%B during 0.5min, 50%B at 2.51 min, stop time at 2.6 min. Post time: 0.5 min.*

*Agilent 6490 Triple Quadrupole MS:*
*Scan type: MRM (Mass Hunter Optimizer software used to define MRM parameters).*
*MRM transition: 501.1 → 293.1, dwell time: 200 ms.*
*Resolution: MS1/MS2: Unit/Unit (Q1: 0.7 m/z / Q2: 0.7 m/z) or Enhanced/Unit (Q1: 0.4 m/z / Q2: 0.7 m/z)*
*Polarity: positive*
*Parameters: Drying gas temperature: 250°C, Drying gas flow: 20 L/min, Sheath gas temperature: 300°C, Sheath gas flow: 12 L/min, Nebulizer pressure: 25 psig, Nozzle voltage: 500 V, Capillary voltage: 3000 V.*

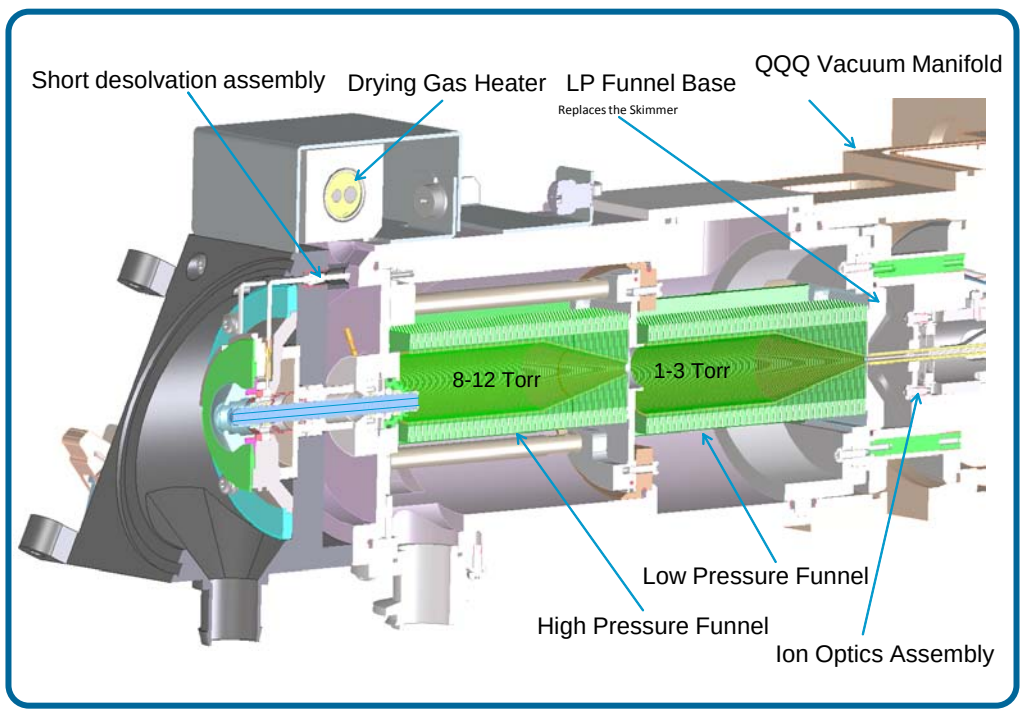
## Experimental

*Agilent 6490 Triple Quadrupole MS technology:*
*The experimental set up consists of a triple quadrupole instrument equipped with new iFunnel technology.*
*The iFunnel technology consists of Agilent Jet Stream technology coupled to a hexabore capillary array and dual ion funnel. The hexabore capillary design allows for maximum ion transmission. The wider radial spread, compared to a single-bore capillary, is compensated by the coupling of the capillary assembly to the dual ion funnel. This ensures that a significantly larger number of ions are transferred to the vacuum stages of the mass spectrometer. Transmission is accomplished by the application of RF confining fields superimposed on an axial-drift DC fields.*
*The new 6490 also has a new curved hexapole collision cell, which also significantly reduces the overall instrument footprint.*

### Hexabore capillary



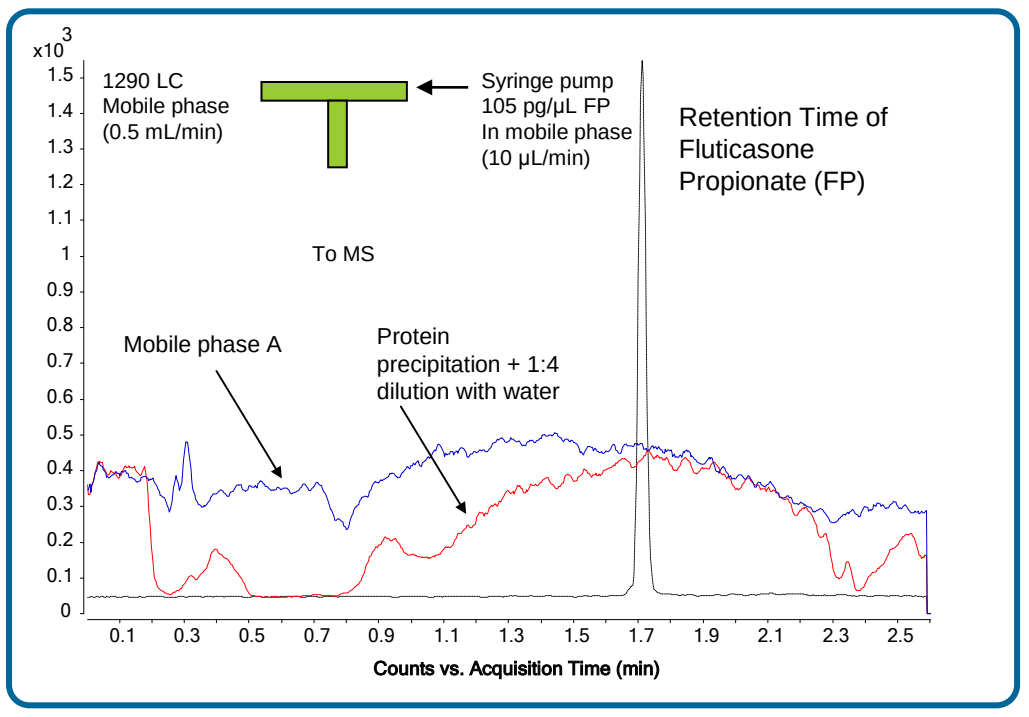

### Dual ion funnel assembly



## Results and Discussion

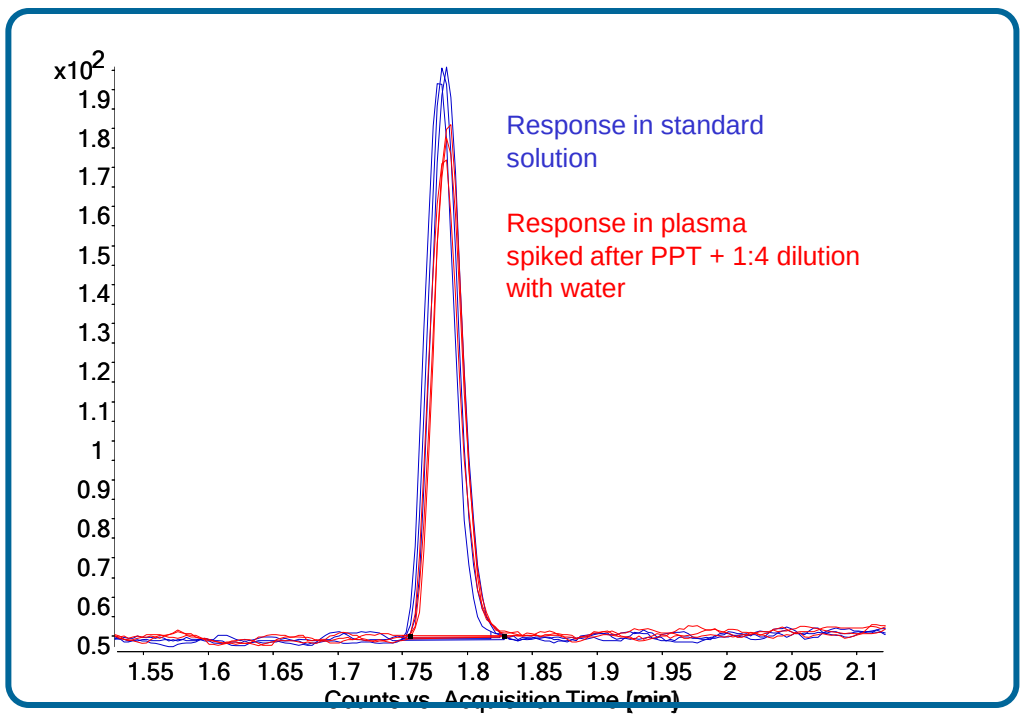
*Qualitative assessment of matrix effects using post-column infusion*

*We have used post column infusion to identify chromatographic regions affected by ionization suppression. Mobile phase A or blank plasma extracts were injected into the UHPLC system. A fluticasone propionate solution was continuously infused post-column and mixed with the column effluent through a tee-junction before entering the mass spectrometer. The MRM chromatogram shown in the figure below show that Fluticasone propionate does not elute in the ionization suppression region.*



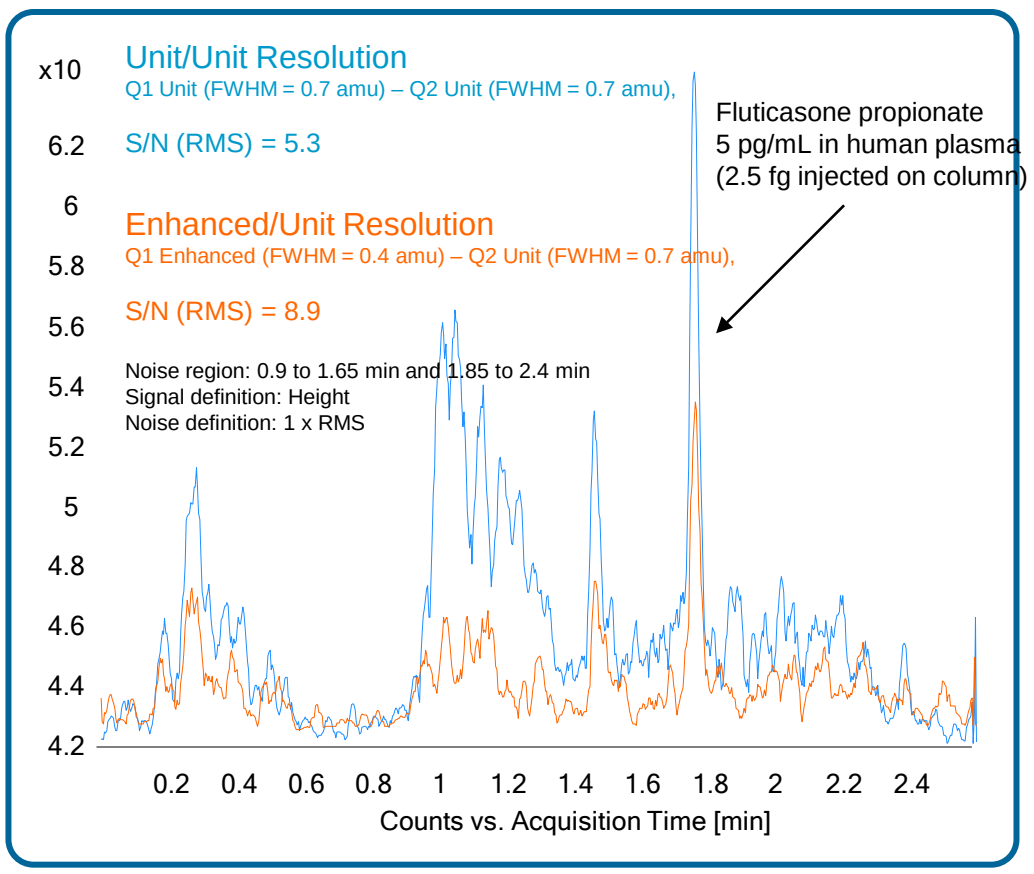

*Quantitative assessment of matrix effects*

*Matrix effects were quantitatively assessed as follows: % matrix effects = [Response FP in plasma spiked after PPT / Response FP in standard solution -1] x 100. The composition of both samples was the same. % of matrix effects > 0 indicates ionization enhancement, < 0 indicates ionization suppression. Calculated % of matrix effects was less than 13%, which is well within acceptance criteria.*




*Evaluation of enhanced resolution capability*

*We have characterized the capability of the Agilent 6490 Triple Quadrupole Mass Spectrometer for MRM-based quantitation in enhanced resolution mode (Q1 FWHM = 0.4 m/z and Q2 FWHM = 0.7 m/z). The enhanced resolution capability of the instrument may be beneficial in eliminating interference peaks and therefore increasing specificity. For 5 pg/mL FP in human plasma (2.5 fg on column), a signal-to-noise (S/N, 1 RMS) ratio of 5.3 or 8.9 was obtained for MRM chromatograms at unit/unit or enhanced/unit resolution, respectively.*
*Absolute signals obtained at unit/unit resolution were about twice as much compared with enhanced/unit settings. Yet, the enhanced/unit setting gave better S/N due to the marked decrease in noise level.*




*Evaluation of sensitivity, linearity, precision and accuracy at enhanded/unit and unit/unit resolution*

*Assay performance (correlation coefficient for calibration curves, precision and accuracy) under unit/unit and enhanced/unit resolution were comparable and both within current pharmaceutical and regulatory assay guidelines. Correlation coefficients ($R^2$) at unit/unit or enhanced/unit resolution were 0.9997 and 0.9998, respectively. Area RSDs were less than 8.7% at both resolution settings. Average accuracy values were within 80-120% both at unit and enhanced resolution modes. The lower limit of quantitation (LLOQ) was 5 pg/mL, corresponding to 2.5 fg abs. injected on-column in both resolution modes.*

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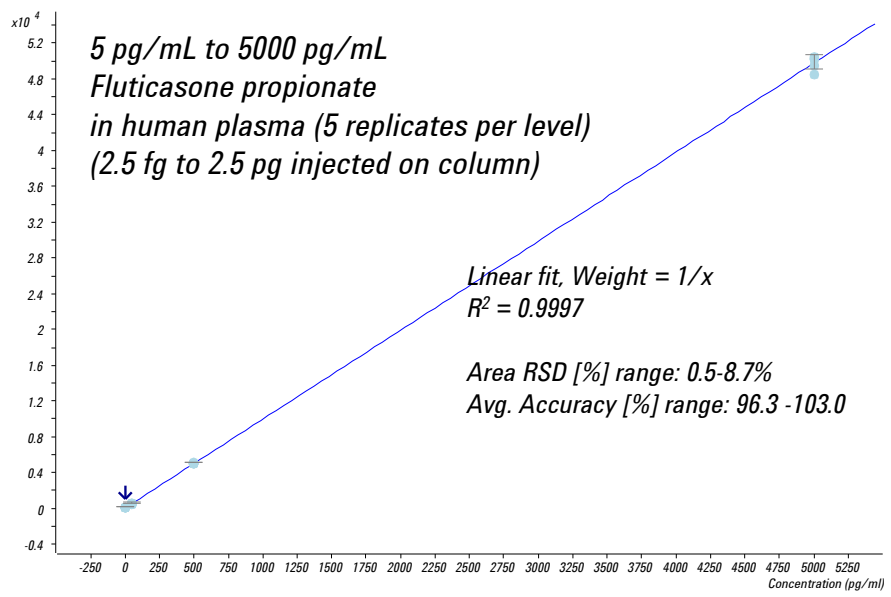
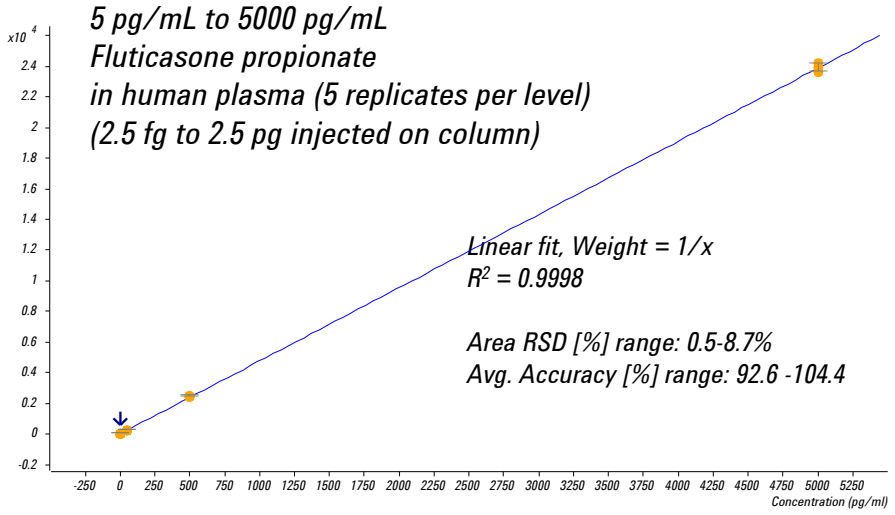
## Results and Discussion

*Calibration curves, area RSDs and accuracy using unit/unit or enhanced/unit resolution*

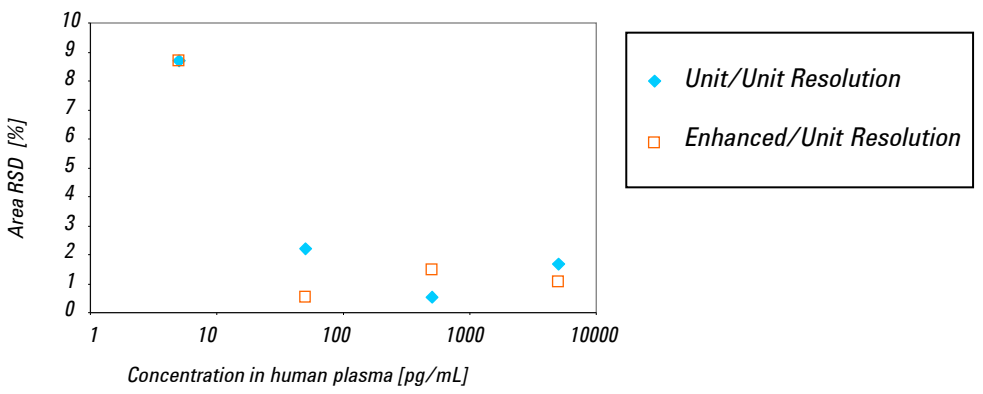
### Unit/Unit resolution



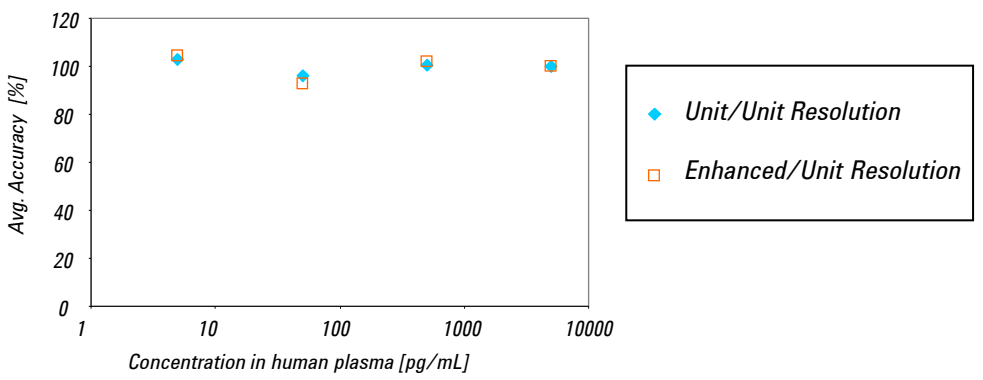

### Enhanced/Unit resolution




### Area RSDs [%]

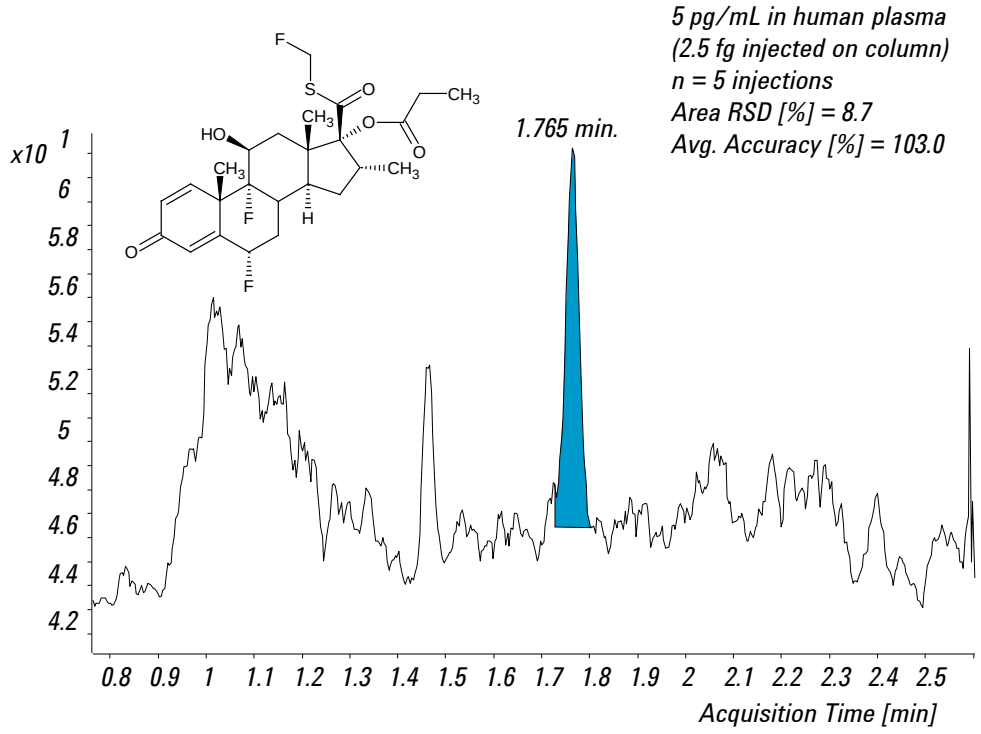



### Average Accuracy [%]

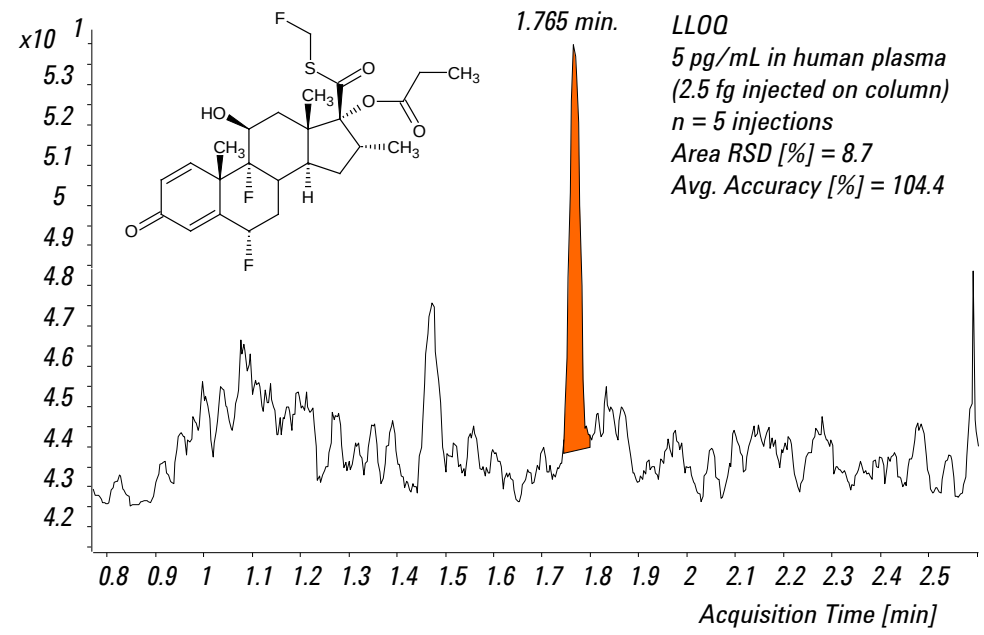



*Lower limit of quantitation at unit/unit or enhanced/unit resolution*

### Unit/Unit resolution




### Enhanced/Unit resolution




## Conclusions

- *Taking full advantage of 6490 Triple quadrupole increased sensitivity, it was possible to streamline the workflow by relaxing the requirements on sample preparation*
- *Sensitivity of the instrument was demonstrated with LLOQ at 5 pg/mL, corresponding to 2.5 fg abs. injected on column*
- *The new enhanced resolution capability of the instrument may be beneficial in eliminating interference noise and therefore increasing specificity.*
- *Assay performance (correlation coefficient for calibration curves, precision and accuracy) under unit/unit and enhanced/unit resolution were similar and both within current pharmaceutical and regulatory guidelines*