

Linearity of an ACQUITY UPLC-Quattro Premier System for the Analysis of 17-Hydroxyprogesterone in Protein-Precipitated Plasma

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Abstract

In this application note, we show the Waters ACQUITY UltraPerformance LC System coupled with the Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer in the analysis of 17-hydroxyprogesterone in protein precipitated plasma.

Introduction

Atmospheric chemical ionization (APCI) is often cited as the technique of choice for improved linearity in quantitative LC-MS/MS. In this application note, we show the Waters ACQUITY UltraPerformance LC System coupled with the Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer in the analysis of 17-hydroxyprogesterone in protein precipitated plasma. The ACQUITY UPLC System runs at high pressure (up to 15,000 psi), resulting in very fast chromatography while maintaining excellent chromatographic resolution. This allows for excellent data quality with good linearity across large calibration ranges. A calibration curve was plotted over five orders linear dynamic range, indicating that a larger range of drug dosage can be analyzed in biological studies.

Experimental

A standard solution of 17-hydroxyprogesterone was made in methanol (1 mg/mL). Subsequent dilutions were made in human plasma to generate a calibration curve from 0.5–100000 pg/μL. The calibration standards were then protein-precipitated by adding acetonitrile (2:1 acetonitrile:plasma); the resultant mixture was centrifuged (ca 3000 rpm, 10 min) and the supernatant taken for analysis by LC-MS/MS. Duplicate injections were made of each standard.

LC Conditions

LC system:	Waters ACQUITY UPLC
Column:	Waters ACQUITY BEH C ₁₈ , 2.1 x 50 mm, 1.7 μm particles

Solvents: A - 70% water, 30% methanol (10mM ammonium acetate, 0.005% acetic acid)

B - 100% methanol (10mM ammonium acetate 0.005% acetic acid)

Injection volume: 5 μ L

Gradient

Time	%A	%B	Flow rate (mL/min)
0.5	65	35	0.6
3.0	10	90	0.6
4.0	10	90	0.6
4.5	65	35	0.6

MS Conditions

MS system: Waters Micromass Quattro Premier

Ion mode: APCI+ve

Corona: 8 μ A

Cone voltage: 40 V

Collision energy: 18 eV

Detection mode: MRM (331.3 > 108.9)

Dwell: 0.3 seconds

Collision gas:

Argon (3.2×10^{-3} mbar)

Results and Discussion

The plot of peak area against concentration showed good linearity over the range of 5–50000 pg 17-hydroxyprogesterone on column. The calibration line was plotted using a linear fit with $1/x^2$ weighting and gave a correlation coefficient of >0.99 (Figure 1).

The QuanLynx browser shown in Figure 1 also demonstrates that all the calibration points gave back-calculated values within $\pm 10\%$ of the theoretical concentrations.

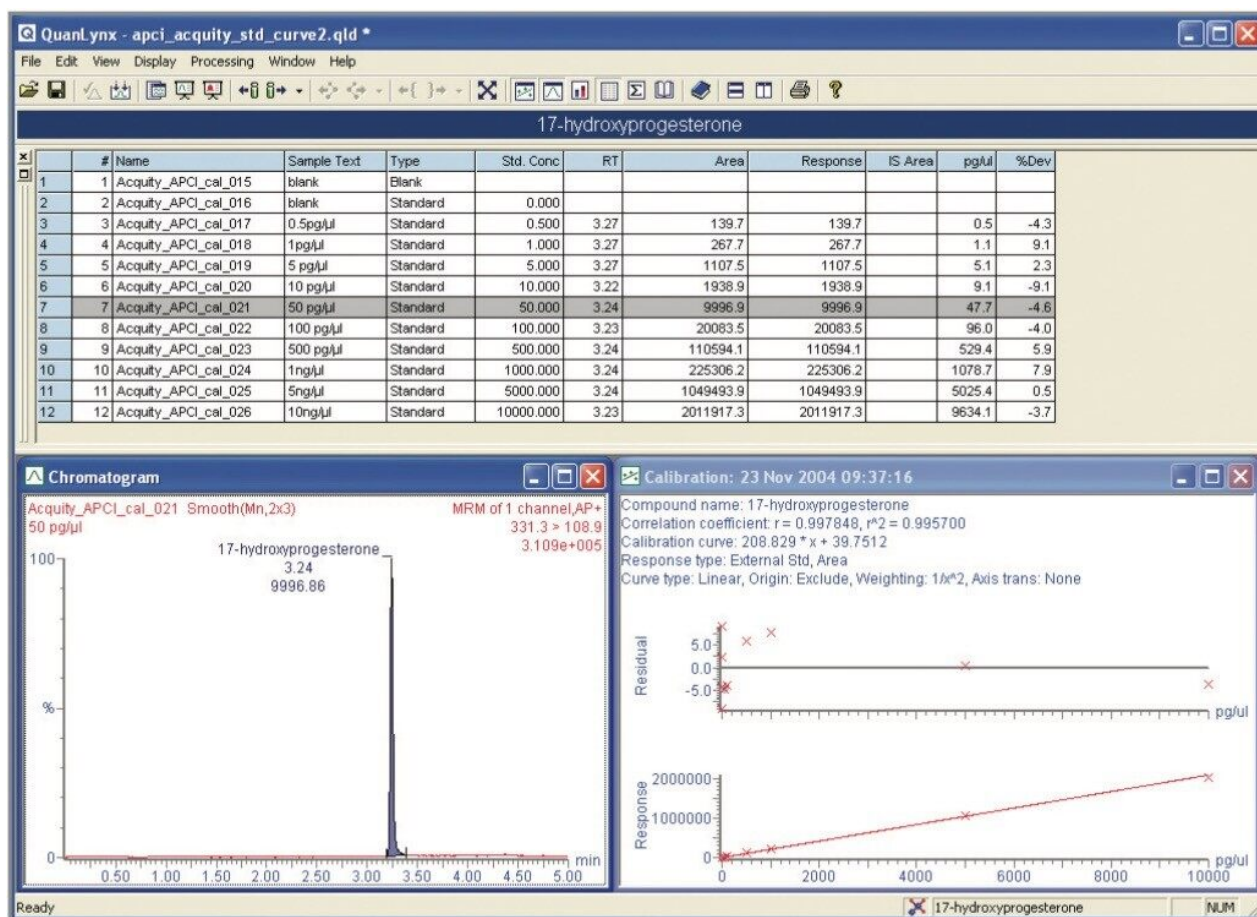


Figure 1. QuanLynx browser showing calibration curve linearity and percent deviations for each standard.

Conclusion

The Waters ACQUITY UPLC System with the Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer has been developed for quantitative LC-MS/MS. The results show that excellent linear dynamic range can be obtained for pharmaceutical compounds in complex biological matrices. Advances in the chromatography have resulted from the improved resolving power and speed of the ACQUITY UPLC System. The results also show that the ACQUITY UPLC with the Quattro Premier can be used to quantify 17-hydroxyprogesterone over five orders of linear dynamic range, i.e. 5–500000 pg on column.

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